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TABLE OF CONTENTS

RAFFEL, DANIEL, and MULLER, H. J., Position effect and gene divisibility considered in connection with three strikingly similar scute mutations 541

GREEN, M. M., and OLIVER, C. P., The action of certain mutants upon the penetrance of heterozygous vestigial wing in *Drosophila melanogaster* 584

COOPER, D. C., and BRINK, R. A., Somatoplastic sterility as a cause of seed failure after interspecific hybridization . . . 593

DEMEREC, M., Genetic behavior of euchromatic segments inserted into heterochromatin 618

SUTTON, EILEEN, Terminal deficiencies in the X chromosome of *Drosophila melanogaster* 628

CLELAND, RALPH E., Analysis of wild American races of Oenothera (Onagra) 636

BRODY, GEORGE, The time of effect of some second chromosome lethals in *Drosophila melanogaster* 645

Index to Volume 25 651

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POSITION EFFECT AND GENE DIVISIBILITY CONSIDERED IN CONNECTION WITH THREE STRIKINGLY SIMILAR SCUTE MUTATIONS[1]

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BACKGROUND OF THE PRESENT WORK

AFTER the proposal of the subgene theory by SEREBROVSKY, DUBININ and their co-workers (SEREBROVSKY and DUBININ, 1929 *et seq.*), in interpretation of the relationships observed by them among scute alleles, an attempt was made by MULLER and co-workers, first at the University of Texas laboratory and later at the Institute of Genetics of the U.S.S.R., to obtain further scute alleles and to analyse these and the ones previously obtained elsewhere, with a view to further testing the above theory and to further investigation of the curious relationship which he had observed between "gene mutations" and "gene rearrangements" (MULLER 1930a, b, 1932). It was soon evident that a high proportion of the scute mutations involved the breakage and reattachment of the X chromosome very close to the locus of scute, but it was not clear whether the phenotypic change represented a "gene mutation" that was near to but, theoretically at least, separable from the rearrangement, or whether it represented the "position effect" of the gene rearrangement itself.

We shall not describe here the work of analysing the position of breakage of these rearrangements of the scute region with respect to each other, reported by MULLER and PROKOFYEVA (1934, 1935a) and briefly mentioned below (p. 566). Suffice it here to say that the numerous rearrangements, although all appearing to possess one point of breakage and interchange of gene connections in one of four definite positions near scute had their other point of breakage anywhere in the X or other chromosomes, so that the gene arrangements occurring in the neighborhood of scute, after the breakages and reattachments had taken place, were in all cases very different from those in the normal chromosome and (with the exceptions to be noted presently) from each other. Correspondingly there was very much diversity in the phenotypic expression of the 'alleles,' although no fixed relation was discernible between the map position of the different

[1] The authors wish to acknowledge their indebtedness to Dr. N. I. Valivov for the provision of the facilities and opportunities for this work. Practically all of the present paper was written in 1938, its publication having been delayed by circumstances connected with the remoteness of the authors from one another.

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chromosome breaks and the kinds of scute phenotype associated with them.

Evidence was, however, obtained that a strong relationship did exist between the type of rearrangement and the type of phenotypic change when two scute mutations that had not previously been studied were subjected to analysis. These were "scute-L8," found by H. LEVY in Texas in 1932, and "scute-S1," found by T. G. SINITSKAYA in Leningrad in 1934. It was found in genetic investigations of the senior author that these two mutants had their X chromosomes broken at sensibly the same place in the scute region, and had them broken also in the right-hand chromocentral ("inert") region of the X, the part of the chromosome between these two breaks being inverted with respect to the rest. In all these particulars, moreover, these two rearrangements agreed with the already known inversion found in the mutant scute-4. (Tests made later did, however, show some difference in the position of the breaks in the chromocentral region, that in scute-4 being to the left of bobbed and block A, that in scute-L8 between them, and that in scute-S1 to the right of both.) By themselves, these coincidences would not be so very striking, since, in the first place, scute mutations (already known often to be associated with breaks near the scute locus) were being searched for, and since, secondly, breaks in the chromocentral region occur relatively frequently. What made the findings striking was the fact that, to parallel this unusual resemblance in type of gene rearrangement, there was also a similar strong resemblance between the types of phenotypic expression of all three of them. In fact, the phenotypic expressions of all three were more like one another than any of them was like any of the numerous other scute mutations known, just as the gene arrangements were more like one another than any of them was like that of any other scute.

These facts were first known only for scute-L8 and scute-4, and were taken by MULLER and PROKOFYEVA (1934, 1935a) as furnishing, along with other facts, strong evidence for their conclusion that the phenotypic changes caused by the so-called mutations accompanying chromosomal rearrangements are in general, except in so far as they are due to losses or duplications, manifestations of a "position effect" on the genes lying near the points of breakage. The later results on scute-S1, agreeing with those on scutes-4 and -L8, seemed to remove all possibility of the agreement between gene arrangement and phenotype being a matter of coincidence.

In view of the significance of such a relationship for our understanding of mutations, both spontaneous and induced, and in order to learn something more concerning the nature of the so-called position effect, it was decided to make a more accurate study of the phenotypic effects of these

three scute mutations, in stocks that were as nearly isogenic as possible. For, despite the phenotypic similarity of these three mutations, certain differences had been observed between them, as well as peculiarities of the recombinational forms, as for example the sterility of crossover males with the left part of the scute-4 and the right part of the scute-L8 chromosomes. These had not been subjected to quantitative study in the earlier work because it was not yet known to what extent they might be due to differences in environmental conditions or in other genes (modifying factors that might lie anywhere in the X chromosome or the autosomes), and to what extent the differences were localisable in and near the gene for scute itself and the right-hand points of breakage.

The expression of scute is known to be rather sensitive to differences in environic factors and modifying genes, and there was no reason to regard the original stocks of the three inversions as containing fewer "invisible" differences in their genes—either in their X chromosomes or autosomes—than most stocks do. In fact, scute-S1 was known to carry a smaller inversion, morphologically somewhat similar to the known inversion called delta 49, in its X chromosome, entirely included within the larger inversion. This same stock of scute-S1 was also known to have an inherited tendency to nicking at the ends of the wings. All this made it the more important to make a comparison on groups of flies which had been made isogenic and in which the effects of environmental differences were minimized. The present paper gives a report of this study on these three scutes. In addition, the effects were studied of the various possible combinations having the left part of one of the inversions and the right part of another, in stocks similarly isogenic.

The results of this investigation show that the phenotypic expressions of these three mutations are, even when subjected to this more exact comparison, very similar to one another but that they are not identical. It is found that both the left and right regions containing the points at which the breaks and recombinations occurred (hereafter referred to as the left and right "ends" of the inversions) appear to affect the development of the bristles. However, while the influences of the left regions (including the "locus of scute" itself) are unquestionable and regular, analysis of the right regions shows them to be much weaker, more uncertain and inconsistent, and it is possible that they do not represent any real effect of the right ends themselves.

The above findings raise important questions of interpretation, concerning the position effect, gene divisibility, etc., which are herein discussed in the light of theoretical considerations and of related work on these subjects.

MATERIALS AND METHODS

In order to obtain differences in the expression of the scute mutations in as nearly as possible identical genetic associations, the chromosomes containing the three original inversions were subjected to crossing so as to replace the whole central portion of the chromosomes, extending from some point between the left point of breakage and the locus of white to a point between the locus of carnation and the right point of breakage (more than 80 percent of the length of the chromosome as seen in the salivary gland cells), by the corresponding region derived from a given X chromosome containing certain mutant genes (apricot, w^a, locus 1.5, miniature, *m*, 36.1, and carnation, *car*, 62.5) that served as markers. The obtaining of such isogenic X chromosomes was especially difficult in the case of scute-S1 because of the initial presence in this chromosome of the smaller inversion, included within the larger one, which greatly reduced crossing over on both sides of it and especially to the right of it. The reconstructed and as nearly as possible isogenic X chromosomes were then introduced into a stock that was isogenic for all the genes in the major portions of the two long autosomes, and thus males were obtained that were isogenic for all genes except those in the small chromosome, IV, and near the very ends of the other chromosomes.

The isogenic X chromosomes were obtained by the series of crosses shown below. In the notation used here the females are represented first except where otherwise stated. The crosses of generations that follow one another are designated by successive numbers, and letters are added to distinguish different lines of descent. Individuals deriving from any given cross either immediately or, in the case of the various stocks made in the course of this work, through a succession of crosses of the same kind, are designated by an "F" in parenthesis with the number of that cross or stock as a subscript. The notations L and R are used to denote respectively the left and right ends of the inversions that had been associated with the mutations designated. These letters are used only in cases where, as a result of crossing over, the two ends of a given inversion are derived from different original inversions.

1. *y sc-4 B*×*car*
2. (F_1)*y sc-4 B/car*×*y sc-4 B*
3. *sc-8 m* w^a×(F_2)*y sc-4 car*
4. (F_3)*sc-8 m* w^a/*y sc-4 car*×*sc-8 m* w^a
5. $\widehat{y}\widehat{y}$×a single (F_4)*y sc-4L car m* w^a *sc-8R* (stock)
6a. *sc-L8/w lz*^{s} dl 49×(F_5) *y sc-4L car m* w^a *sc-8R*
7a. (F_{6a})*sc-L8/y sc-4L car m* w^a *sc-8R*×*y sc-13 w*
8a. (F_{7a})*y sc-4L car m* w^a *sc-L8R/y sc-13 w*×*y w lz*^{s} dl 49

9a. (F_{8a})*y sc-4L car m* w^a *sc-L8R/y w lz*^{s} dl 49×*y w lz*^{s} dl 49 (stock)
8b. $\widehat{y}\widehat{y}$×(F_{7a})*sc-L8L car m* w^a *sc-8R* (stock)
9b. *y sc-4*×(F_{8b})*sc-L8L car m* w^a *sc-8R*
10b. (F_{9b})*y sc-4/sc-L8L car m* w^a *sc-8R*×*y sc-13 w*
11b. (F_{10b})*sc-L8L car m* w^a *sc-4R/y sc-13 w*×*y w lz*^{s} dl 49
12b. (F_{11b})*sc-L8L car m* w^a *sc-4R/y w lz*^{s} dl 49×*y w lz*^{s} dl 49 (stock)
13b. (F_{12b})*sc-L8L cur m* w^a *sc-4R/y w lz*^{s} dl 49×(F_5)*y sc-4L car m* w^a *sc-8R*
14b. (F_{13b})*sc-L8L car m* w^a *sc-4R/y sc-4L car m* w^a *sc-8R*×*y sc-13 w*
15b. (F_{14b})*y sc-4 car m* w^a*/y sc-13 w*×*y w lz*^{s} dl 49
16b. (F_{15b})*y sc-4 car m* w^a*/y w lz*^{s} dl 49×*y w lz*^{s} dl 49 (stock)
10c. (F_{9a})*y sc-4L car m* w^a *sc-L8R/y w lz*^{s} dl 49×(F_{8b})*sc-L8L car m* w^a *sc-8R*
11c. (F_{10c})*y sc-4L car m* w^a *sc-L8R/sc-L8L car m* w^a *sc-8R*×*y sc-13 w*
12c. (F_{11c})*sc-L8 car m* w^a*/y sc-13 w*×*y w lz*^{s} dl 49
13c. (F_{12c})*sc-L8 car m* w^a*/y w lz*^{s} dl 49×*y w lz*^{s} dl 49 (stock)
6d. *sc-S1/w lz*^{s} dl 49×(F_5)*y sc-4L car m* w^a *sc-8R*
7d. (F_{6d})*sc-S1/y sc-4L car m* w^a *sc-8R*×*y sc-13 w*
8d. (F_{7d})*sc-S1L car m* w^a *sc-8R* male×$\widehat{y}\widehat{y}$ (stock)
9d. (F_{9a})*y sc-4L car m* w^a *sc-L8R/y w lz*^{s} dl 49×(F_{8d})*sc-S1L car m* w^a *sc-8R*
10d. (F_{9d})*y sc-4L car m* w^a *sc-L8R/sc-S1L car m* w^a *sc-8R*×*y sc-13 w*
11d. (F_{10d})*sc-S1L car m* w^a *sc-L8R/y sc-13 w*×*y w lz*^{s} dl 49
12d. (F_{11d})*sc-S1L car m* w^a *sc-L8R/y w lz*^{s} dl 49×*y w lz*^{s} dl 49 (stock)
17a. (F_{16b})*y sc-4 car m* w^a*/y w lz*^{s} dl 49×(F_{8d})*sc-S1L car m* w^a *sc-8R*
18a. (F_{17a})*y sc-4 car m* w^a*/sc-S1L car m* w^a *sc-8R*×*y sc-13 w*
19a. (F_{18a})*sc-S1L car m* w^a *sc-4R/y sc-13 w*×*y w lz*^{s} dl 49
20a. (F_{19a})*sc-S1L car m* w^a *sc-4R/y w lz*^{s} dl 49×*y w lz*^{s} dl 49 (stock)
13e. (F_{12b})*sc-L8L car m* w^a *sc-4R/y w lz*^{s} dl 49×(F_{7d})*y sc-4L car m sc-S1R*
14e. (F_{13e})*y sc-4L car m sc-S1R/y w lz*^{s} dl 49×*y w lz*^{s} dl 49 (stock)
15e. (F_{14e})*y sc-4L car m sc-S1R/y w lz*^{s} dl 49×(F_{8d}) *sc-S1L car m* w^a *sc-8R*
16e. (F_{15e})*y sc-4L car m sc-S1R/sc-S1L car m* w^a *sc-8R*×*y sc-13 w*
17e. (F_{16e})*sc-S1 car m* w^a*/y sc-13 w*×*y w lz*^{s} dl 49
18e. (F_{17e})*sc-S1 car m* w^a*/y w lz*^{s} dl 49×*y w lz*^{s} dl 49 (stock)
19e. (F_{18e})*sc-S1 car m* w^a*/y w lz*^{s} dl 49×(F_5)*y sc-4L car m* w^a *sc-8R*
20e. (F_{19e})*sc-S1 car m* w^a*/y sc-4L car m* w^a *sc-8R*×*y sc-13 w*
21e. (F_{20e})*y sc-4L car m* w^a *sc-S1R/y sc-13 w*×*y w lz*^{s} dl 49
22e. (F_{21e})*y sc-4L car m* w^a *sc-S1R/y w lz*^{s} dl 49×*y w lz*^{s} dl 49 (stock)
23e. (F_{22e})*y sc-4L car m* w^a *sc-S1R/y w lz*^{s} dl 49×(F_{8b})*sc-L8L car m* w^a *sc-8R*
24e. (F_{23e})*y sc-4L car m* w^a *sc-S1R/sc-L8L car m* w^a *sc-8R*×*y sc-13 w*
25e. (F_{24e})*sc-L8L car m* w^a *sc-S1R/y sc-13 w*×*y w lz*^{s} dl 49
26e. (F_{25e})*sc-L8L car m* w^a *sc-S1R/y w lz*^{s} ld 49×*y w lz*^{s} dl 49 (stock)

Having thus made up stocks of the three inversions and of all six possible recombinations of their ends, so constituted that the whole central region of each of these nine X chromosomes between the loci of white and carnation, inclusive, was the direct descendant of the same original *y sc-4L car m* w^a *sc-8R* chromosome, it was necessary to introduce each of these nine X chromosomes into a stock that had been made isogenic for the two long autosomes. This stock had been prepared by the series of crosses shown below. In what follows "II" and "III" represent those normal second and third chromosomes which entered into the composition of the isogenic stock. The presence of other normal chromosomes is indicated only by blank spaces in the appropriate positions.

1f. *Cy sp/apl*×*ru h D* CXF *ca/rucuca*
2f. (F_{1f})*Cy sp/* ; *ru h D* CXF *ca/*×*apl/* ; *rucuca/*
3f. (F_{2f})*Cy sp/apl*; *ru h D* CXF *ca/rucuca* (stock)
4f. $\widehat{yy}$; II/ ; III/×(F_{3f})*Cy sp/apl*; *ru h D* CXF *ca/rucuca*
5f. (F_{4f}) single female $\widehat{yy}$; *Cy sp*/II; *ru h D* CXF *ca*/III
×(F_{3f})*Cy sp/apl*; *ru h D* CXF *ca/rucuca*
6f. (F_{5f})$\widehat{yy}$; *Cy sp*/II; *ru h D* CXF *ca*/III
×(F_{5f})*Cy sp*/II; *ru h D* CXF *ca*/III
7f. (F_{6f})$\widehat{yy}$; II; III×II; III (stock, called below the "M" stock)

Here *CXF* represents a combination of inversions in the third chromosome effectively preventing all crossing over except a small amount near the ends. It lies in the same chromosome with the dominant marker "Dichaete" (*D*). The obtaining of this useful combination involved a series of steps carried out with the intention of obtaining such a result. After preliminary radiations of Dichaete by MULLER, which had yielded only some inversions permitting considerable crossing over, a much better combination, called CX, was obtained by OLIVER in Texas in 1929; and further irradiation of the latter chromosome, first by STONE in Texas and then by FYODOROVA in Leningrad in 1935 (see D.I.S., 6), resulted in the present CXF combination.

"*rucuca*" represents a well-known combination of third chromosome mutant genes consisting of: roughoid (*ru*), 0.0; hairy (*h*), 26.5; thread (*th*), 42.2; scarlet (*st*) 44.0; curled (*cu*), 50.0; stripe (*sr*), 62.0; sooty (e^s), 70.7; and claret (*ca*), 100.7.

"*Cy*" represents Curly, which is a spontaneous dominant mutation in the second chromosome associated with two inversions, one suppressing crossing over throughout the major portion of the left arm of the second chromosome and the other throughout the major part of the right arm.

"*apl*" represents a well-known combination of second chromosome mutant genes consisting of: aristaless (*al*), 0.0; dumpy (*dp*), 13.0; black

(*b*), 48.5; purple (*pr*), 54.5; curved (*c*), 75.5; plexus (*pl*), 100.5; and speck (*sp*), 107.0.

The stock, "M," obtained in the cross "7f" was homozygous for all the genes in the long second and third chromosomes except possibly those near the very ends. The nine scute-containing X chromosomes isogenic for all but their end regions were now brought into association with the second and third chromosomes of stock "M" by means of a series of crosses, all of the type defined below. There were nine such series, begun with females derived from F9a, F12b, F12d, F13c, F16b, F18e, F20a, F22e, and F26e, respectively. In what follows, the nine X chromosomes from these stocks, containing any of the three original scute mutations or the six recombinations of the ends of the inversions associated with them, are designated simply as "scute."

27. "scute"/*y w lz*^{s} dl 49 × (F_{31})*Cy sp*/*apl*; *ru h D* CXF *ca*/*rucuca*
28. (F_{27})"scute"/; *Cy sp*/; *ru h D* CXF *ca*/ × (F_{71})II; III (stock "M")

By breeding together flies from the above cross (28), heterozygous for the long autosomes of the "M" stock (F_{71}) and the crossover-suppressing chromosomes marked by *Cy* and *D*, (that is, flies showing Curly and Dichaete but not roughoid, hairy, claret, or speck), males with the desired X chromosomes and homozygous for the second and third chromosomes of the "M" stock were produced. It was found, however, that such males were too inviable to appear in sufficient numbers for the purpose of this investigation. To obviate this difficulty females obtained from cross (28) were mated with males from the homozygous stock from Amherst which PROFESSOR PLOUGH kindly furnished us with and which is referred to below as stock "A." The final cross involving each of the nine isogenic lines then was

29. (F_{28}) "scute"/; *Cy sp*/IIM; *ru h D* CXF *ca*/IIIM × the normal stock "A."

These crosses yielded males (F_{29}) "scute"; IIM/IIA; IIIM/IIIA.

To recapitulate, then, males of this kind were obtained with each of the nine kinds of X chromosomes marked with w^{a}, *m*, and *car*, to ensure that the X chromosomes, at least between the loci of *w* and *car*, as well as the two long autosomes, except for their very ends, would be the same in all of the flies of all nine types of scute combinations.

In order to minimize the differences in cultural conditions, the crosses yielding all these nine final types (F_{29}) were made simultaneously, in vials containing food derived from the same lot, and each of these nine crosses was at the same time made in replicate, in a series of ten or more vials. The bristles on both sides of approximately 25 males of each of the nine

types, derived in approximately equal proportions from all the ten cultures of a given type, were recorded (thus giving approximately fifty sets of bristles in each case).

RESULTS

Classes of effects on the bristles

In table 1 we present the average number of bristles of each kind for the nine types of F_{29} males studied, together with the standard errors of these averages as calculated by dividing the standard deviations found by $\sqrt{n}$, n being the number of flies on which the average was based. The first three columns give the results for the three original inversions, scute-4, scute-S1, and scute-L8, and the last six columns give the results for the various possible recombinational types. An inspection of these columns will show that although these three scute mutations and their combinations are strikingly similar to one another and indeed much more so than to any other scute mutations hitherto described, yet no two of the nine types seem to have exactly the same phenotypic expression and, so far as the three original types are concerned, wherever significant differences in the frequency of given bristles occur the order of frequency (from higher to lower bristle number) is always scute-4, scute-S1, scute-L8.

Several bristles were not significantly changed from the normal number by any of these three inversions or recombinations of them, or the change was so slight that the values of all the nine groups might be regarded as virtually random deviations from the same nearly-normal figure. Among these bristles were the internal verticals, the posterior dorsocentrals (except for some slight fluctuations), and the posterior sternopleurals. The same applies to the thoracic microchaetes. All of these except perhaps the posterior sternopleurals belong to the "achaete" group; that is, they do not ordinarily seem to be absent as a result of loss or change of the scute locus, but rather of the neighboring locus of achaete.

Other bristles were entirely absent in all nine types of scutes, or were absent in some types and appeared in the others in such very small numbers that the differences between the frequencies observed in any of the types had little or no statistical significance. The bristles so greatly reduced as this were the post-verticals, the external verticals, the internal postalars, the two humerals, the two scutellars, the coxals, the ventrals, the first and third orbitals, and the external supraalars. The last, however, in just one group, that of scute-S1, had a bristle frequency such that a deviation nearly as great as twice its own standard error would have been required to give a value of zero. There are three other bristles that belong in nearly the same class as the preceding, except that each of them deviates more widely from the zero frequency than allowed on the above

scheme in just one of the nine groups of flies. These bristles are the second orbitals (deviating in scute-4L·scute-L8R), the presuturals (also deviating in scute-4L·scute-L8R) and the anterior notopleurals (deviating in scute-4L·scute-S1R).

There is another group of bristles—the ocellars, the sternopleural microchaetes and the middle sternopleurals—which are partially reduced in frequency but, unlike most bristles that are partially reduced, are affected to sensibly the same degree in all nine groups—or, in the case of the middle sternopleurals in all but one of the nine groups, with the one exceptional case representing only a slight deviation from the rest.

Except for the isolated and not very marked cases of deviation above mentioned—which can hardly be used for the drawing of definite conclusions regarding the effects of left and right ends—the significant differences in bristle frequencies are largely confined to the other bristles, and we are therefore grouping these more significant bristles together in the tables so as to facilitate comparison. These bristles include the following: the anterior dorsocentrals and the internal supraalars (these as well as the internal verticals, posterior dorsocentrals, and thoracic microchaetes mentioned above are the chaetae which are especially affected by changes involving the locus of achaete, to the left of that of scute); the external postalars, the posterior notopleurals, the anterior sternopleurals, the dorsal abdominal microchaetes and macrochaetes and the three sets of sternitals. The latter we may group together for consideration, as the variations of all three sets of sternitals proved to be consistently parallel. For the reasons above given, we shall in what follows confine our attention to the bristles of these eight types—or ten types, when we take the three types of sternitals separately. The values found for them are given in the first ten rows of the tables.

Comparison of effects of the original inversions

Considering the frequencies of the latter bristles only in the first three columns of table 1 we see that all three of these original inversions, although on the whole strikingly similar to one another and indeed much more so than to any other scute mutation previously described, have their own distinctive frequencies for these particular bristles in the great majority of cases. The so-called achaete bristles mentioned above (anterior dorsocentrals and internal supraalars) were not affected by the scute-4 inversion, were barely significantly reduced by the scute-S1 inversion and were reduced nearly to one half their normal frequency by the scute-L8 inversion. The extent of reduction of the external postalars, like that of the achaete bristles, was, in order of increasing reduction: scute-4, scute-S1, scute-L8, the reduction in the step from the first to the

TABLE 1

Bristle frequencies for each scute combination.

CLASSES OF BRISTLES	BRISTLES	SC-4	SC-S1	SC-L8	SC-4L · SC-S1R	SC-4L · SC-L8R	SC-S1L · SC-4R	SC-S1L · SC-L8R	SC-L8L · SC-4R	SC-L8L · SC-S1R
1	dorsocentrals (ant.)	2.00±0.00	1.86±0.06	1.39±0.13	2.00±0.00	1.96±0.04	2.00±0.00	1.91±0.06	1.70±0.11	1.52±0.09
	supraalars (int.)	2.00±0.00	1.86±0.06	1.46±0.09	2.00±0.00	1.93±0.05	1.81±0.10	1.86±0.07	1.70±0.10	1.62±0.07
	postalars (ext.)	1.61±0.12	1.34±0.11	1.14±0.11	1.88±0.16	2.00±0.00	1.56±0.12	1.55±0.18	1.07±0.15	1.16±0.10
	notopleurals (post.)	1.89±0.06	0.80±0.14	0.68±0.13	2.00±0.00	2.00±0.00	1.31±0.17	1.36±0.15	0.37±0.10	0.56±0.09
	sternopleurals (ant.)	2.00±0.00	1.34±0.13	1.07±0.15	2.00±0.00	1.71±0.08	1.25±0.14	1.73±0.10	0.60±0.15	0.98±0.11
	sternitals (seg. 2)	5.14±0.15	2.83±0.21	1.48±0.22	4.70±0.15	4.50±0.16	2.75±0 29	2.23±0.24	2.33±0.21	2.18±0.19
	sternitals (seg. 3)	5.18±0.29	3.03±0.19	1.32±0.19	4.76±0.16	4.93±0.17	2.50±0.25	2.09±0.23	2.50±0.19	2.26±0.17
	sternitals (seg. 4)	5.47±0.16	2.89±0.25	1.93±0.23	4.87±0.20	4.72±0.16	3.25±0.24	2.41±0.23	2.83±0.19	2.18±0.16
	dorsal abd. micro.	+	=	=	−	−	=	=	=	=
	dorsal abd. macro.	−	=	o	−	=	=	o+	=	o+
2	ocellars	1.86±0.06	1.71±0.10	1.68±0.10	1.71±0.16	1.29±0.15	1.69±0.15	1.73±0.12	1.70±0.11	1.50±0.33
	sternopleurals (macro)	0.14±0.07	0.11±0.05	0.18±0.09	0.08±0.09	0.18±0.09	0.25±0.14	0.32±0.13	:	0.16±0.06
	sternopleurals (micro)	10.36±0.30	10.63±0.24	10.47±0.31	10.78±0.32	10.39±0.19	10.63±0.35	10.86±0.75	9.83±0.20	10.40±0.20
3	orbitals (1st)	:*	0.03±0.03	:	0.12±0.08	0.07±0.05	:	0.05±0.04	:	0.04±0.03
	orbitals (2nd)	0.11±0.08	0.09±0.05	0.14±0.06	0.18±0.09	0.89±0.15	0.13±0.09	0.05±0.04	:	0.02±0.02
	orbitals (3rd)	:	0.09±0.05	:	:	0.04±0.04	0.06±0.06	:	:	:
	postverticals	:	0.03±0.03	:	:	0.04±0.04	:	0.09±0.06	:	:
	verticals (ext.)	:	:	:	:	:	:	:	:	:
	supraalars (ext.)	:	0.09±0.05	:	:	:	:	:	:	:
	postalars (int.)	:	:	:	:	:	:	:	:	:
	presuturals	:	0.09±0.05	:	0.06±0.06	0.21±0.09	:	.09±0.06	:	:
	humerals (dors.)	:	:	:	:	:	:	:	:	:
	humerals (vent.)	:	:	:	:	:	:	:	:	:
	notopleurals (ant.)	0.11±0.06	0.17±0.12	:	0.65±0.17	:	:	:	:	0.06±0.03
	scutellars (ant.)	:	0.03±0.03	:	:	:	:	:	:	:
	scutellars (post.)	:	:	:	:	:	:	:	:	:
	coxals	:	:	:	:	:	:	:	:	:
	ventrals	:	:	:	:	:	:	:	:	:
4	verticals (int.)	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.07±0.05	2.00±0.00	2.00±0.00	2.00±0.00	2.02±0.04
	dorsocentrals (post.)	2.00±0.00	2.00±0.04	2.07±0.07	2.00±0.00	2.00±0.00	2.13±0.08	2.00±0.00	2.17±0.08	1.92±0.07
	sternopleurals (post.)	1.89±0.06	1.97±0.03	1.86±0.07	1.94±0.06	1.96±0.04	2.00±0.00	2.00±0.00	1.80±0.09	1.88±0.06
	dors. thor. micro.	+	+	+	+	+	+	+	+	+

* : designates 0.00±0.00. 1. Differentially reduced. 2. Non-differentially reduced. 3. Maximally reduced. 4. Minimally reduced.

second being greater. The reduction of the posterior notopleurals was in the same order, although here the differences between scute-S1 and scute-L8 were not significant. The anterior sternopleurals show the above order of reduction very distinctly. The three groups of sternitals, all of which were in all three types reduced to less than half their normal frequencies, again show very distinctly the above order of reduction (scute-4 > scute-S1 > scute-L8 —where by the sign > we mean "has more bristles than." The most obvious differences that could be seen on inspection were to be found in the microchaetes and macrochaetes of the dorsal surface of the abdomen. These were hardly affected by scute-4, the microchaetes appearing normal and only a few of the macrochaetes being absent. In the other two scutes the abdominal macrochaetes were almost entirely lacking and fewer than half of the abdominal microchaetes were present.

It may be added that the occasional nicking of the wing which had been observable in the original scute-S1 stock did not occur in the reconstructed scute-S1 stock isogenic with those of scute-4, scute-L8, etc. Unless this nicking was suppressed by the presence of the gene for miniature or by some unsuspected mutant gene in the isogenic stock, it had therefore been an expression of some genetic abnormality not associated with the scute-producing inversion (but perhaps associated with the smaller inversion).

Mean effects of the left ends of the inversions

The average numbers of the different bristles in the various classes of males having recombinations of the ends of the inversions are given in the last six columns of table 1. Further consideration of these may be postponed, however, until we have made a more generalized comparison of the effects of the different left ends and also of the different right ends. To facilitate such a comparison, tables 2 and 3 have been constructed. In table 2 we have averaged together the results from all three classes of flies having the left end of each given inversion. Thus, for determining the effect of the left end of the scute-4 inversion we have averaged the results from the chromosomes having the composition *sc-4L·sc-4R*, *sc-4L·sc-S1R* and *sc-4L·sc-L8R*. In this way we have eliminated, so far as possible, differential effects of the right ends of the inversions on the expression of the scute character, leaving for comparison the effects of the left ends in the material as a whole.

Examination of the more significant rows (the first ten) of table 2, representing the bristles showing differential reduction in frequency, shows that these three different left ends are responsible for considerable differences in the expression of scute, differences usually comparable in magnitude to those shown in the first three columns of table 1, where the

TABLE 2

Frequencies for different left ends (with results for different right ends averaged together).

CLASSES OF BRISTLES	BRISTLES	LEFT END OF SC-4	LEFT END OF SC-S1	LEFT END OF SC-L8
1	dorsocentrals (ant.)	1.99±0.01	1.90±0.04	1.54±0.06
	supraalars (int.)	1.97±0.02	1.85±0.04	1.60±0.04
	postalars (ext.)	1.82±0.05	1.45±0.08	1.13±0.07
	notopleurals (post.)	1.96±0.02	1.08±0.10	0.54±0.06
	sternopleurals (ant.)	1.90±0.04	1.44±0.08	0.90±0.08
	sternitals (seg. 2)	4.80±0.10	2.66±0.14	2.03±0.12
	sternitals (seg. 3)	5.01±0.10	2.66±0.14	2.08±0.12
	sternitals (seg. 4)	5.01±0.11	2.84±0.15	2.29±0.11
	dor. ab. mi.	−	=	=
	dor. ab. ma.	=	=	=
2	ocellars	1.60±0.08	1.71±0.08	1.60±0.06
	sternopleurals (ma.)	0.16±0.05	0.21±0.06	0.12±0.04
	sternopleurals (mi.)	10.47±0.16	10.70±0.08	10.26±0.12
3	orbitals (1st)	0.06±0.03	0.03±0.02	0.03±0.01
	orbitals (2nd)	0.41±0.08	0.08±0.03	0.05±0.01
	orbitals (3rd)	0.01±0.01	0.06±0.03	:
	postverticals	0.01±0.01	0.04±0.02	:
	verticals (ext.)	:*	:	:
	supraalars (ext.)	:	0.04±0.02	:
	postalars (int.)	:	:	:
	presuturals	0.10±0.04	0.07±0.04	:
	humerals (dors.)	:	:	:
	humerals (vent.)	:	:	:
	notopleurals (ant.)	0.19±0.05	0.08±0.04	0.03±0.02
	scutellars (ant.)	:	0.01±0.01	:
	scutellars (post.)	:	:	:
	coxals	:	:	:
	ventrals	:	:	:
4	verticals (int.)	2.03±0.02	2.00±0.00	2.01±0.02
	dorsocentrals (post.)	2.00±0.00	2.03±0.03	2.03±0.04
	sternopleurals (post.)	1.93±0.03	1.99±0.01	1.85±0.04
	dor. thor. micro.	+	+	+

* : designates 0.00±0.00.

1. Differentially reduced.
2. Non-diffly. reduced.
3. Maximally reduced.
4. Minimally reduced.

individuals have the original inversions and hence differ in regard to both ends at once.

Moreover, as in the case of the whole original inversions, but with even greater regularity, whenever significant differences in bristle number

occur the left end of scute-4 produces the least deviation from normal, the left end of scute-S1 an intermediate amount of deviation, and that of scute-L8 the greatest. Thus, so far as the left ends alone are concerned, as well as, with less regularity, for the original inversions as wholes, scute-L8 is the most extreme allele and scute-4 the least extreme. Of course, since scutes are "hypomorphic" mutations, the mutant genes have effects on the character—bristle production—similar to but weaker than those of the normal gene. This means that scute-4 really has the strongest effect—the most normal—and scute-L8 the weakest, on the developmental processes whereby the bristles are formed, although by a kind of inversion of speech scute-4 might be referred to as the "weakest allele" (in the sense of giving the least difference from the normal) and scute-L8 as the "strongest allele."

Examining the bristle effects of the left ends in more detail, in the case of the group of bristles subject to differential reduction,[2] we see not only that, as in the case of the original inversions taken as wholes, the order of increasing reduction was scute-4, scute-S1, scute-L8, but also that the differences in all of these cases were significant. This applied to all eight types of bristles in question, namely: the anterior dorsocentrals, the internal supraalars, the external postalars, the posterior notopleurals, the anterior sternopleurals, the sternitals, and the dorsal abdominal macro- and microchaetes. These differences were on the whole even greater, and showed more regularity, than those found in the case of the original inversions, but they were throughout in the same direction.

Mean effects of the right ends

The same kind of investigation was made of the effects of the right ends of these three inversions. Table 3 was constructed in the same manner as was table 2 except that in this case the three groups of individuals having the same right ends were in each case averaged together in order to obtain as nearly as possible the average effects of the right ends alone. An examination of table 3 will show in the first place that the right ends, or factors associated with them in some of the series of the present experiments, undoubtedly affect the bristles differentially, but, secondly, that these effects are seldom nearly so strong as the differential effects of the different left ends. The bristles most strongly affected by right-end differences are the posterior notopleurals and the abdominal bristles (both dorsal and sternital), but none of these effects, even, are as marked as those of the left ends on these same bristles. Thirdly, in the case of the right ends

[2] As before noted, the second orbitals, presuturals and anterior notopleurals are exceptional in being reduced to zero or nearly zero in all but one of the nine classes of flies, but in all three of these cases this class contains the left end of the scute-4 inversion.

no regularity in the effects of the three different inversions, such as was found in the case of their left ends, is apparent. Seldom if ever do we find the same sequence in the order of decrease from normal bristle number for the right ends as for the left. Neither do we find the same sequence

TABLE 3

Frequencies for different right ends (with results from different left ends averaged together).

CLASSES OF BRISTLES	BRISTLES	RIGHT END OF SC-4	RIGHT END OF SC-S1	RIGHT END OF SC-L8
1	dorsocentrals (ant.)	1.88±0.04	1.72±0.06	1.74±0.06
	supraalars (int.)	1.84±0.05	1.76±0.04	1.74±0.05
	postalars (ext.)	1.38±0.09	1.34±0.07	1.56±0.07
	notopleurals (post.)	1.15±0.10	0.88±0.08	1.35±0.09
	sternopleurals (ant.)	1.27±0.10	1.27±0.08	1.49±0.08
	sternitals (seg. 2)	3.49±0.20	2.82±0.14	2.76±0.20
	sternitals (seg. 3)	3.51±0.19	2.96±0.14	2.83±0.21
	sternitals (seg. 4)	3.92±0.16	2.83±0.14	3.06±0.19
	dor. ab. mi.	—	=	=
	dor. ab. ma.	=	=	=
2	ocellars	1.76±0.06	1.61±0.06	1.55±0.08
	sternopleurals (ma.)	0.11±0.04	0.15±0.04	0.22±0.06
	sternopleurals (mi.)	10.20±0.06	10.53±0.14	10.55±0.15
3	orbitals (1st)	:*	0.05±0.02	0.04±0.02
	orbitals (2nd)	0.07±0.04	0.07±0.03	0.39±0.08
	orbitals (3rd)	0.01±0.01	0.03±0.02	0.01±0.01
	postverticals	:	0.01±0.01	0.04±0.02
	verticals (ext.)	:	:	:
	supraalars (ext.)	:	0.03±0.02	:
	postalars (int.)	:	:	:
	presuturals	:	0.04±0.02	0.10±0.04
	humerals (dors.)	:	:	:
	humerals (vent.)	:	:	:
	notopleurals (ant.)	0.04±0.02	0.20±0.05	:
	scutellars (ant.)	:	0.01±0.01	:
	scutellars (post.)	:	:	:
	coxals	:	:	:
	ventrals	:	:	:
4	verticals (int.)	2.00±0.00	2.01±0.02	2.03±0.02
	dorsocentrals (post.)	2.10±0.04	1.96±0.04	2.03±0.03
	sternopleurals (post.)	1.88±0.04	1.92±0.04	1.94±0.03
	dors. thor. mi.	+	+	+

* : designates 0.00±0.00.

1. Differentially reduced.
2. Non-diffly. reduced.
3. Maximally reduced.
4. Minimally reduced.

of bristle reduction for the different right ends when different bristles are compared. In the cases of a few bristles such as the second orbitals the order of the effects of the three inversions appears to be reversed for the right ends as compared with the left, but in most cases some order of differential reduction of bristles is to be found for the right ends quite different from that for the left, and the orders for the different bristles disagree with one another.

In more detail the results presented in table 3 show the following—limiting our consideration first to the eight types of bristles which are clearly affected differentially in more than one of the nine genetic classes of flies of table 1: The internal supraalars and the external postalars were nearly equally reduced in their frequencies although not entirely absent in all the groups with the three different right ends. The other six types of bristles were affected differentially by the three right ends. The anterior sternopleurals and the posterior notopleurals showed a somewhat greater average frequency in the males carrying the right end of scute-L8 than in those with other right ends. (The same was true of the presuturals and the second orbitals, but these bristles belong to the rather anomalous category in which only one of the nine genetic classes differed significantly from a near-zero value.) In respect to these two types of bristles, then, the two ends of the scute-L8 inversion acted as though more or less complementary to one another, that is, while the left end caused greater bristle reduction than other left ends the right end caused lesser reduction than other right ends. Here it might be imagined that the bristle reducing effect had merely been apportioned differently between left and right ends in scute-L8 as compared with the other two inversions. When, however, we compare the results for scute-4 and scute-S1, in respect of these same bristles, we find that, for the anterior sternopleurals, the differences between the effects of the right ends of the scute-4 and scute-S1 inversions were not significant, unlike what was found for the left ends of these inversions. For the posterior notopleurals, the right end of scute-S1 gave a greater reduction than that of scute-4, so that there was no question of a complementary action of these two right ends in relation to the corresponding left ends.

Moreover, quite the opposite of a complementary action was to be seen in the case of the other four bristles—the anterior dorsocentrals, the microchaetes and macrochaetes of the dorsal abdominal surface and the sternitals—for all these were most numerous in males with the right end of scute-4, just as, in studying the left ends, we had found them most numerous with the left end of this same inversion. In the groups having the other two right ends, these bristles were about equally reduced—a

result that was again at variance with the differential effects shown on these types of bristles by the left ends of scute-S1 and scute-L8.

The degree of consistency of the effects of the different left ends

Let us now examine our data with a view to determining to what extent the averages given in tables 2 and 3 really represent the relative effects produced by the different left or right ends (as the case may be) regardless of what other ends they are combined with. For example, we have seen in table 2 that (when we average together the results for different right ends) the posterior notopleurals occur in significantly higher frequency in flies with the left end of scute-4 than in those with the left end of scute-S1 and in significantly higher frequency in the latter, in turn, than in those with the left end of scute-L8, the relative numbers of posterior notopleural bristles thus giving, for the left ends of the scute inversions, the order 4>S1>L8. But although this holds true in table 2, where the results from chromosomes having all three different right ends but the same left end are averaged together, we may ask, Does this same relation hold also in the presence of each specific right end in turn? This may be determined by turning to table 1 and first comparing the results for the posterior notopleurals (row 4) in the first, sixth and eighth columns of data, all columns in which the right end is derived from scute-4. Here we find again the relation 4>S1>L8. Examining next the fourth, second and ninth columns, in which the right end of scute-S1 is present, we once more find the relation 4>S1>L8. Finally, comparing the third, fifth and seventh columns, having the right end of scute-L8, we see that here too the 4>S1>L8 rule prevails. Thus the results for the posterior notopleurals shown in table 2 represent a consistent tendency, a difference in the effects of these left ends that occurs no matter what right end is present with them, so long as the latter is held constant.

To facilitate a more general examination of the same question with reference to the effects of the different left ends, on different bristles, table 4 has been constructed. This gives the order of bristle number of the three left ends, first, as found in table 2, where the right ends were averaged, and then as found in the presence of each kind of right end considered separately, for all bristles of the differential class.

It will be seen from table 4 that in the great majority of cases the results obtained in the presence of the different right ends agree fairly well with one another in showing the same order of reduction of bristles by the different left ends in the presence of different right ends, and hence that they agree also with the table 2 averages. Of course where, in the presence of a particular right end, nearly maximal or minimal numbers (2 or 0) occur for more than one left end, this does not disprove a greater tendency

to bristle production on the part of one of these left ends than of the other, and so it cannot be taken as inconsistent with a difference shown in the results for the averages. In the case of a few bristles, however—notably the posterior dorsocentrals—there is less hindrance to production of excess bristles and here "2" cannot be regarded as definitely maximal, if (as seems likely here) an intensification of the same processes which cause the production of one bristle (instead of none) in a given limited region of the integument tends to cause, further, the production of two instead of one. But in the case of most bristles the latter process, if possible, requires

TABLE 4

Order of normality with respect to bristle production shown by different left ends.

	WITH ALL RIGHT ENDS AVERAGED TOGETHER	WITH RIGHT END OF SC-4	WITH RIGHT END OF SC-S1	WITH RIGHT END OF SC-L8	RATING AS TO AGREEMENT
dorsoc. (ant.)	4>S1≫L8	4*=S1>L8	4>S1>L8	4≧S1≫L8	good
su. al. (int.)	4>S1≫L8	4>S1≧L8	4>S1>L8	4≧S1≫L8	good
p. al. (ext.)	4≫S1>L8	4≧S1≫L8	4>S1≧L8	4>S1>L8	good
nt. pl. (post.)	4≫S1≫L8	4≫S1≫L8	4≫S1≧L8	4≫S1≫L8	excellent
st. pl. (ant.)	4≫S1≫L8	4≫S1>L8	4≫S1>L8	4=S1≫L8	good
sterni. (2)	4≫S1>L8	4>S1≧L8	4≫S1>L8	4≫S1>L8	good
sterni. (3)	4≫S1>L8	4≫S1=L8	4≫S1>L8	4≫S1>L8	good
sterni. (4)	4≫S1>L8	4≫S1=L8	4≫S1>L8	4≫S1≧L8	good
d. ab. mi.	4>S1=L8	4>S1=L8	4>S1=L8	4≫S1=L8	excellent
d. ab. ma.	4>S1=L8	4>S1=L8	4≫S1>L8	4>S1>L8	good

= denotes equality or virtual equality of bristle number.
≧ denotes an inequality which has little statistical significance.
> denotes a nearly significant or significant inequality.
≫ denotes a great inequality of high statistical significance.
* Signifies that the numbers for the groups on both sides of the equality sign are maximal, and that for this reason differences in the effects of the ends in question could not be observed.

considerably more "causative pressure" than the former, except where qualitative changes are involved as an aid to it. It is also to be expected, as a result of errors of sampling, that some differences seen in averages will appear as virtual equalities in the smaller records given by the separate right ends. Bearing these considerations in mind, perusal of table 4 will show that there is on the whole a rather good agreement in the order of effectiveness of the left ends obtained in the presence of different right ends.

In fact, only the ocellars (which in general show a partial reduction but one not markedly differential) show any marked inconsistency in the results, and the results from them are hardly to be considered as being so reliable as those from the other bristles anyway. For the ocellars, unlike

the other bristles, vary not merely by presence or absence but also in size, so that it is often hard to say whether the bristle is present or not, and the values given for them are not so objective. An additional sign of their irregularity is the fact that the rather arbitrary values that have been found for the ocellars do not follow, even in the averages for the left ends, the order 4>S1>L8 found for all bristles which are clearly affected differentially.

For the bristles here under consideration the order 4>S1>L8, varied occasionally by equality of adjacent classes, was found in nearly every case, in the presence of each right end considered separately, as well as for the right ends averaged together. Thus there can be no doubt, first, that in the case of each bristle there is a general rule representing the order of effectiveness of these left ends on that bristle (regardless of the right end) and, second, that this order of effectiveness is the same for bristles of nearly all kinds.

The degree of consistency of the effects of the different right ends

The orders of frequency of the differential bristles found for the different right ends, in the presence of the different left ends, are shown in table 5, which has been constructed by the same method as table 4. It will be seen that the effects of the right ends show little if any consistency. That is, for any given bristle, the order of effectiveness of the three right ends in the presence of one left end bears little or no relation to their order in the presence of another left end, and the order shown for a given bristle in the averages (as in table 3) thus represents no real rule or general tendency for the right ends, in acting on that bristle.

It is, to be sure, not to be expected that a given gene, or, still better, group of genes, in comparison with its alleles or group of alleles, will necessarily affect the production of all bristles in the same direction, and in fact two mutant scute alleles themselves often show, in the case of different bristles, an opposite order of effectiveness. But even in such cases we should expect that, in the presence of a given modifier (corresponding, for purposes of this illustration, to a given left end), a given pair of alleles would usually have differential effects that were similar, so far as they were discernible at all, to those which it had when in the presence of another modifier. For, in the gene interactions of development, additive effects of some degree (grading into complementary effects at times) represent much the more frequent types of processes, and cases of "negative interaction" (when a given gene-difference in the presence of one allele of another locus gives an effect opposite in direction to that which it has in the presence of a different allele of the latter locus) are very rare, as is

also to be expected theoretically. Thus the great amount of disagreement (including much "negative interaction") here found would indicate that the apparently diverse effects of these right ends have in reality been caused by other factors, no doubt of a genetic nature, but independent of the right ends themselves and differing in different stocks having the same right end. Differences of this kind might exist in the more distal parts of

TABLE 5

Order of normality with respect to bristle production shown by different right ends.

	WITH ALL LEFT ENDS AVERAGED TOGETHER	WITH LEFT END OF SC-4	WITH LEFT END OF SC-S1	WITH LEFT END OF SC-L8	RATING AS TO AGREEMENT
dorsoc. (ant.)	4≧L8=S1	4=S1=L8	4=S1=L8	4≧S1≧L8	good
su. al. (int.)	4≧S1=L8	4=S1=L8	4=S1=L8	4=S1≧L8	fair
p. al. (ext.)	L8≧4=S1	L8≧S1≧4	4=L8≧S1	S1=L8=4	very bad
nt. pl. (post.)	L8≧4>S1	4*=S1=*L8	L8=4>S1	L8=S1≧4	bad
st. pl. (ant.)	L8≧4=S1	4*=S1>L8	L8>S1=4	L8=S1>4	bad
sterni. (2)	4>S1=L8	4≧S1=L8	S1=4>L8	4=S1>L8	fair
sterni. (3)	4>S1=L8	4=L8=S1	S1≧4=L8	4=S1>L8	fair
sterni. (4)	4>L8=S1	4>S1=L8	4=S1=L8	4>S1=L8	fair
d. ab. mi.	4>S1=L8	4>S1=L8	4=S1>L8	4=S1>L8	fair
d. ab. ma.	4>S1=L8	4=S1>L8	4=S1>L8	4>S1>L8	fair

(Symbols as in table 4.)

* Mc mal; see footnote table 4.

the major autosomes and in the fourth chromosomes of the isogenic lines first constructed, and in the chromosomes of the inbred Amherst line to which the former were crossed.

The suspicion that it is not the differences in the right ends themselves, or at any rate not genes there derived from the right end of the original normal X chromosome, that are responsible for the phenotypic differences that are manifested so irregularly in the presence of the different right ends, is heightened by the consideration that the finding of genes influencing bristle pattern at all, in precisely this location in the right end of a normal X chromosome, would be entirely unexpected—although we might attribute the effects to genes of a hypothetical "scute complex" that have been transferred to this position from the scute region by the inversions. In the absence of other, more noticeable, effects of the differences in the right ends of the inversions, it would be strange if exactly that character, scute, which we are studying because of the known influence upon it of the different left ends, should also be capable of being affected differentially by the genes derived from the given very limited region of the right end of the X chromosome. This consideration has especial pertinence in view

of the fact that the region in question is part of the so-called "inert" or "chromocentral region," and that in large measure the chromocentral regions of other chromosomes, also, act as if homologous to it, so that most phenotypic differences which it might be responsible for, even if they did happen to concern bristle pattern, would in fact have been "covered" or obscured, in the males examined, by the presence of the Y and the other chromosome parts in question.

These objections would not apply, however, to the hypothesis that the differences in the right ends of the inversions were due to a gene or genes affecting bristle pattern that had originally been in the left end, and had formed part of some hypothetical "scute complex," as above mentioned. But even this hypothesis still suffers from the important difficulty that the supposed effects of these genes are so irregular and non-additive, and often even negative in their interaction. In addition to that, there are other difficulties, which can be better appreciated after we consider separately the two forms which this hypothesis may assume.

On the first form of the hypothesis, exactly the same amount of bristle-producing gene material has been transferred from the left end to the right by all three inversions, but differs in its effect in the three cases by reason of the "position effect" of the differences in gene associations, attendant upon the presence or absence, and different degree of propinquity (as well as, perhaps, arrangement with regard to each other) of bobbed, block A, and/or other, "invisible" genes, originally of the right end. If, however, we admit that the bristle-producing region of the original left end is thus divisible into at least three parts capable of having some effect on bristles even when separated from one another[3] (namely, "achaete," "scute," and the genetic material in question that became transferred to the right end), it would seem arbitrary to assume that the divisibility stopped with this, and so it would not appear improbable that the amount of bristle-producing material, capable of autonomous action on bristles, which was transferred from left to right end, differed in the different cases, and that these differences resulted in a part at least of the apparent phenotypic differences between the right ends.

The latter would represent the second form of the hypothesis for accounting for the right-end differences as representing real effects of the present right-ends themselves. This form of the hypothesis, however, suffers from another important difficulty, besides that of the irregular, non-additive character of the effects already mentioned. That is, there

[3] This is already partially implied by the explanation of the scute mutations as position effects involving the separation of scute from genes to the right of it, but this conception alone does not require the gene or genes to the right to be able to act autonomously on bristles, that is, when removed from the propinquity of the scute locus itself.

should in this case be some tendency to a complementary action of the left and right ends of a given inversion on the production of bristles, for genes for bristle production placed at the right end of a given inversion would necessarily have been taken away from the left end of the same chromosome. As we have seen, however, no such tendency is observable. For example, although the left end of scute-4 is associated with more bristle production than any other left end, the right end of scute-4 does not tend to produce fewer bristles than other right ends, but, in the case of three of the five types of bristles that seem to be affected differentially by the right ends, its effect too is to produce more bristles than other right ends. Moreover, a scheme of the kind in question would lead us to expect that certain combinations should result in maximal bristle production and the complementary classes in minimal, and this too is not found to be the case.

All this does not mean that the hypothesis of a highly divisible "scute complex" is necessarily wrong, but that it is gratuitous in this connection since it will not help to explain the present results concerning the right ends. If it does hold, it must be concluded that probably the elements in the right-hand portion of the scute complex function little or not at all when removed from the main portion to the left of them. In that case, the apparent differences shown by the right-hand ends are due to extraneous genes, only casually associated with the right ends. Thus these differences would not serve as evidence for the divisibility hypothesis, which would have to be founded upon other grounds. And even the first form of the hypothesis, according to which there is only one main separable scute locus (at least, only one of autonomous action) to the right of "scute proper," which gives different right-end effects as a result of the different position effects of bobbed, Block A, etc., on this locus, meets with the difficulty of the irregularity of these effects, and so becomes less probable than the alternative view, that of extraneous factors. It is hoped that a test for the latter factors may be made at some later date by a repetition of our observations on somewhat different "isogenic" stocks of the nine scutes.

THE INCIPIENT GENETIC ISOLATION OF THE SCUTE-4 AND SCUTE-L8 LINES

In the examination of the recombination classes produced by crossing over between the original scutes, an interesting finding concerning fertility was made. It was found that all males tested (ca. 50) of one of these crossover classes—that having the left end of the scute-4 inversion and the right end of scute-L8—were completely sterile, although those of all three original inversions and of all the five other recombination types were fertile. This means that the original mutants, scute-4 and scute-L8, each

contain a different mutant gene, or closely linked combination of genes, which in its original setting, and probably also in an otherwise normal genotype, does not cause sterility, but which, in combination with the gene lying near the other end of the other scute inversion, does act to cause sterility.

These, then, are complementary genes for sterility. They help to illustrate the mechanism of origination of genetic isolation in the evolutionary splitting of species from one another. Had these genes been dominant and autosomal, or dominant and effective in the female, the two original lines of scute would have given sterile F_1 hybrids with one another, by reason of this complementary action. As it is, on account of the recessiveness of the mutations, only a fraction of the F_2 from crosses between the two lines are sterile, so that the inter-sterility here may be considered as only "partial" or "incipient."

The fact that one at least of the complementary genes upon which this sterility depended was not associated with the breakage and reattachment points of either of the inversions emerged after the isogenic lines were obtained (F_3), for the recombination type in question, when modified by having all but the ends of its X chromosome replaced by our standard X (with the markers w^a, *m* and *car*), proved to be as fertile as any of the other scutes. This showed that the inter-sterility was not simply a result of a deficiency or duplication caused by corresponding breaks of the two scutes being in different positions. In other words, the intersterility was genic in origin, not due to the aneuploidy resulting from recombination of different structural types.

By the same token, the result illustrates the desirability of obtaining isogenic lines for the making of exact comparisons between the positions of breakage of different rearrangements, and proves that the prolonged crossings to which our lines had been subjected were not uncalled for.

SIMILARITY OF THE THREE SCUTE MUTATIONS AS EVIDENCE FOR THE POSITION EFFECT

The phenotypic effects of some dozen different "scute" mutations, including among them scute-4, have been carefully studied and recorded by the Moscow geneticists, under the leadership of SEREBROVSKY. All of these were shown to give distinctly different patterns of bristle reduction, and although isogenic lines were not constructed, the amount of agreement found in different lines of the same allele was, in general, enough to show that the differences found between the different scute mutations were not, in the main, due to "modifying factors." Among these twelve "scute" mutations and others studied since that time, the only ones which, like scute-4, have been found to cause a marked reduction of practi-

cally all the so-called "scute" bristles and no or a much lesser reduction of the "achaete" bristles are scute-S1 and scute-L8. Moreover, the relative amounts of reduction of the different bristles are very similar for these three mutations, although, as we have seen, neither the relative nor total amounts of reduction are identical.

Now the fact that the phenotypic patterns of just these two mutations, scute-S1 and scute-L8, are so very similar to that of scute-4, despite all the other scute mutations having patterns so different from them, as well as, usually, from each other, might be attributed to mere coincidence, until the chromosome structure of the various scute mutations is analyzed. These analyses, made mainly by MULLER, show that, among the various other scute mutations, some (such as scutes 1, 3, 5, 6 and 11) show no change of gene arrangement demonstrable either by genetic tests (of segregation and crossing over) or by salivary gland observation, others (such as the inversions scute-10 and scute-J1, and the insertional translocation scute-19) involve minute rearrangements of various kinds, one of the rearrangement (breakage) points of which always lies very close to the scute region, while still others involve divers kinds of rearrangements of larger sections of chromatin, although here too one of the rearrangement points always lies close to the scute region. Among the larger rearrangements there are translocations of various kinds, as in scute-2, scute-C, scute-J4 and scute-S2, and inversions.

It is clear that only the large inversions could have a chromosome structure, in the neighborhood of the scute locus, similar to that of scute-4. These may therefore be examined in more detail. They include (besides scute-4 and the other two inversions with which we have here dealt) scute-7, scute-8, scute-9, and scute-29. Of these, as the studies of MULLER in making recombinations of the chromosomes in question with one another have shown, scutes 7, 9 and 29 involve left-hand breaks close to the scute region, but lying to the right not only of the scute locus itself, but also of another gene, which itself is just to the right of scute, and the absence of which is lethal in its effect. These three left-hand breaks are in sensibly the same position as one another, but the right-hand points of breakage of all three inversions are widely different from one another, being in scute-7 between *rb* and *sn*, in scute-29 between *m* and *f*, and in scute-9 to the left of the proximal chromocentral region but between *car* and a gene which, in haploid dosage (relative to the rest of the X), results in minute bristles (Minute-*n*?). All these gene rearrangements, then, are clearly quite unlike that of scute-4. In the case of scute-8, on the other hand, there is, as in scute-4, one breakage point in the proximal chromocentral region (although it is to the right of the loci of Block A and bobbed), but the other breakage point, that close to the scute locus, is

to the left of the latter, so that this rearrangement results in quite different gene associations and sequences in the neighborhood of scute than does scute-4.

Thus we see that, of all the scute mutations the structure of which is approximately known, only scute-S1 and scute-L8 produce gene configurations in the neighborhood of the scute locus that even remotely resemble that of scute-4. But as for scute-S1 and scute-L8 themselves, their configurations are very similar to that of scute-4, both in respect to the position of the left-hand break and in respect to that of the right-hand break.

In regard to the left-hand point of breakage, it is to be noted that, of all the above mentioned scute mutations, these alone have their left-hand break lying just to the right of the locus of scute, between it and the "lethal" locus above mentioned that lies to the right of scute. And not only do these three left-hand points of breakage all agree in lying between these two genes (or genetic regions?) but, so far as can be determined by recombination tests, these two genes are adjacent to each other, and the three breaks thus lie in identical positions. If they lay in different positions in the case of any two of the chromosomes carrying these inversions, then one of the two recombination chromosomes, produced by crossing over between these two chromosomes, would contain the left end of that inversion which had the break farther to the left and the right end of that inversion which had the break farther to the right. This, then, would be deficient for the small region lying between these two breaks; hence the fact that the breaks were in different positions would be evident from the inviability or abnormal appearance of this crossover class, unless the small region in question contained genes of so little importance that their absence produced neither inviability nor abnormal morphological effects. As a matter of fact, the crossovers are, as we have seen, all comparable in their morphology, viability, fertility, etc., with the three original scute inversions, when the lines are made isogenic.

In this connection, the question might again be raised as to whether or not the differences in bristle frequency seen in some of the recombinational as compared with the three original inversions could in part be expressions of small deficiencies of this kind, for genes (or "sub-genes") that mainly affect bristle pattern. It could further be argued that the effects of such genes on bristle production, and even on viability, might be greater than they might seem to be at first sight, inasmuch as the scute "mutations" themselves had already caused so much reduction of bristles and of viability, of an associated kind, that further effects of the same general sort would not be very evident. All this involves questions to which we must return later. But, whatever the answer to them may be, it is clear that no genes important for life or for the production of a normal morpho-

logical appearance, except, possibly, genes or "sub-genes" connected with bristle pattern itself, can lie between the left-hand points of breakage of scutes-4, S1 and L8.

In regard to the right-hand points of breakage of the three scutes in question, it is to be observed that, as salivary gland observations have shown, all are located within the so-called "inert region," better called the chromocentral or heterochromatic region, of the proximal end of the X chromosome, to the left of the centromere. It is known that genes transplanted into or near to a chromocentral region are particularly apt to undergo changes in their expression associated with their change in position. The salivary observations, combined with genetic and cytological tests (the latter on metaphase chromosomes) of the crossovers between our three inversions have shown that, although these three right-hand points of breakage all lay very near together, and within the chromocentral region, they were all in slightly different positions, that of scute-4 lying to the right of a part of the chromocentral region but to the left of both bobbed and "block A" (the gene responsible for producing most of the metaphase chromatin of this region—see MULLER, RAFFEL, GERSHENSON and PROKOFYEVA 1937), that of scute-L8 lying to the right of bobbed but to the left of block A (a configuration that had probably involved in addition a minute rearrangement of the latter two genes with respect to each other), and that of scute-S1 lying to the right of both these genes. These genes are known to be extremely close together, however, so that the gene associations finally obtaining in the scute region of the reconstituted chromosomes must have been very similar in all three cases, far more alike than any of them was like that of any other known scute, and than any other known scute rearrangements were like one another. Moreover, other results agree with these in indicating that, for a given locus subject to manifold kinds of changes in expression (such as the loci of white, brown and scute), there tends to be a considerable similarity between the changes associated with its transplantation to the neighborhood of a chromocentral region, even though the exact location of the reattachment point may differ somewhat from case to case. This observation, however, is anticipating our argument somewhat.

The important fact for us to note at this point is that, of all the numerous and diverse scute mutations known besides scute-4, it is just those two scute mutations (S1 and L8) phenotypically most closely resembling scute-4 and each other, which at the same time are by far the most like scute-4, and like each other, in regard to their content and arrangement of genes in the neighborhood of the scute locus. This coincidence is a far too special one to be attributed to chance. Yet it must be chance factors, such as just what path given electrons will take and just which chromo-

nemas later happen to approach one another, that determine what new arrangement of genes will emerge from a given irradiation. Now if the kind of phenotypic change concomitantly produced is not a secondary matter, resulting from the kind of gene rearrangement, it cannot plausibly be conceived as having its nature fixed by just those same factors of chromonema movement, etc., which determined just what anatomy the new gene rearrangement should have. Hence the correspondence between the phenotypic similarities of the three scute mutations in question and their similarities of gene rearrangement in the scute region constitute strong evidence of the dependence of their phenotypic expression upon their type of rearrangement. That is to say, the change in expression of these genes in the scute region must be an effect of the change in positions of the genes relative to one another—the phenomenon known as "position effect."

Though not hitherto published in detail, the above was the line of reasoning followed by MULLER and PROKOFYEVA (1934) in reaching the conclusion that scute-4 and scute-L8 were expressions of the "position effect." At that time, however, these mutations had not been studied in detail and scute-S1 was not yet known; the present work, then, supports their conclusion. It may be recalled that this conclusion of MULLER and PROKOFYEVA, which implied that, in the case of the two scute rearrangements in question, "gene mutations" separate from the rearrangements but of simultaneous origin with them had not occurred, had raised presumptive grounds for going further, to the conclusion that the numerous other mutations that accompanied demonstrable rearrangements of the scute region were likewise only expressions of the changes in arrangement, and that a similar process was at work in the case of other loci as well. And, after the discovery of minute rearrangements (MULLER, PROKOFYEVA and RAFFEL 1935) the suspicion even arose that possibly the same phenomenon might even be at work in many (or all?) of the cases where there was no rearrangement large enough to be cytologically or genetically detectable as such. Such an extension of the "position-effect" interpretation of the phenotypic changes, at least to the other cases of demonstrable rearrangements, fitted in with a considerable amount of evidence from other sources, obtained by various workers at about the same time.

THE POSITION EFFECT AS A POSSIBLE CAUSE OF THE OBSERVED DIFFERENCES

Accepting the interpretation that the great phenotypic similarity of the three scutes in question is an expression of the extreme similarity of their gene arrangements, it would seem but logical to attribute those

phenotypic differences which do exist between them to the fact that their gene arrangements are not quite identical. We have seen that all three right-hand breaks differed from one another in their positions with respect to "block A" and the normal allele of bobbed. This would cause the gene groups of both the left and right ends of all three inversions to differ from one another in respect to the presence or absence of these two genes, and so it would provide a basis, through their position effects, for the observed phenotypic differences which undoubtedly distinguish the left ends, and also for any which may really distinguish the right ends of the inversions, although, as we have seen, it is possible that the differences found between the latter may really not be attributable to the right ends at all.

It might be contended that the possibility has not yet been ruled out of the existence of other genes (or gene parts?), besides bobbed and block A, lying between either the different left-hand or the different right-hand points of breakage (or some in each position), and that these other genes may be responsible, in part at least, for the observed phenotypic differences. If this is true, however, these other genes must, all told, occupy an exceedingly small length of the chromosome thread. For PROKOFYEVA'S parallel cytological studies of the salivary chromosomes have shown no perceptible difference between the locations of the three left-hand breaks, all lying at sensibly the same place within the heavy scute-containing band-complex, and they have shown further that the right-hand breaks differ in position by only one or two of the faint lines of the chromocentral region.

The hypothesis of other genes than those already detected (bobbed and block A), lying between either the right or the left-hand breakage points, would also imply that the genetic method used for detecting the existence of genes between the breakage points had not been sufficiently delicate to reveal all of them. This possibility cannot be denied, for it must be recalled that the method discloses the existence only of such genes (or gene-complexes) the deficiency of which causes lethal effects or externally distinguishable morphological abnormalities.

The essence of the method is to obtain both complementary types of recombinations between each two of the gene arrangements to be compared (in these cases, both crossovers between the inversions), and to note in each case which, if either, of these two types is lethal or abnormal. If two breaks are not in just the same position, that recombinant (crossover) having the portions of chromosome to the left of the break that lies farther to the left, and to the right of the break that lies farther to the right, will necessarily be deficient for the small region between the two points of breakage, and if the deficiency for these genes causes detectable abnormality or inviability, the fact that a gene or gene-complex normally

lies between the two points of breakage (and hence too the fact that the first break really is to the left of the second) will thereby be revealed. But not all genes are indispensable for life, that is, not all deficiencies are lethal (see MULLER 1935, PANSHIN 1938), nor is there any reason to suppose that all which are not lethal necessarily cause readily visible abnormalities. And yet such "invisible genes" might, theoretically, be as active as any others in exerting a "position effect" upon neighboring genes, and so might be held responsible for the differences found between the phenotypic effects of the left ends of the scutes here studied—and also between those of the right ends, if we grant the existence of genes affecting bristle pattern at the right end of these inverted chromosomes at all.

In the case of the right-hand breaks of the three scutes here studied, there is an especially strong possibility that other genes than the two discovered may have lain between the positions of breakage. For the region here in question, the so-called "inert" or chromocentral region of the proximal end of the X chromosome, as before noted, is known to be homologous, at least in part, with the Y chromosome, and probably with chromocentral regions of other chromosomes as well. Hence deficiencies of genes in this region would more often be "covered" by the presence of normal alleles of them in the other chromocentral regions, and would accordingly be less apt to be recognizable through the causation of inviability or abnormality. Moreover, it is found empirically (whether or not the above be the reason for it) that deficiencies of chromocentral regions do in fact have less tendency than those of other regions to cause detectable morphological or lethal effects.

To avoid possible confusion here we may point out that while, previously, we used this as an argument against a direct (non-positional) effect of genes from the right end on bristles, we are here using it to show that such genes, undetected because of the lack of such direct effect on bristles or other characters, could nevertheless be present and influence the bristles through their position effects upon the genes, derived from the left end, which especially affect bristle pattern.

The consideration last mentioned does not, of course, apply to the left-hand breaks unless we choose to regard the scute region as one in which one or more extremely minute "repeats" (duplications), closely adjoining one another and similar to but much smaller than that found in the mutant bar eye, have occurred in the relatively recent evolutionary history; for such duplications would tend to have a "covering" effect on deficiencies, similar to that existing in the case of chromocentral regions. The similarity in function of the neighboring loci of achaete and scute makes it likely (MULLER 1935b, SEREBROVSKY 1938), that there has at some time been some duplication of this kind, but as achaete is to the left

of scute and these breaks to the right of it, additional duplications would have to be postulated for our cases, if we would suppose the existence between the different left-hand breaks of undetected genes, covered, or partially covered, in the above manner. It is quite conceivable, however, that even if there were no duplications having such a "covering" action, the deficiencies might have had effects too small to have been revealed by the methods used—especially if we indulged in speculation to the effect that deficiencies might be of lesser length than that commonly envisaged as the probable (but, more correctly, the estimated maximal) length of a gene.

On either the supposition of minute duplications involving scute, or the related one of different positions of breakage within lesser lengths than that attributed to the scute gene or gene-complex as a whole, differences in bristle production could be brought about not only by the position effect of such minute parts, as ordinarily conceived, but also in a more direct way, by the presence or absence of these parts. For this and other reasons, a proper evaluation of the meaning of our results now involves us in considerations of the degree of divisibility of the genetic material, the criteria by which we recognize the presence of genes and their spatial or numerical limits, and the extent to which the string of genes may be regarded as discontinuous or continuous in structure and functioning.

CONSIDERATIONS CONCERNING THE DIVISIBILITY OF THE GENETIC MATERIAL

It may be recalled that all the methods of estimating gene number and size have admittedly given merely a minimal figure for the former and a maximal for the latter, with no indication of what the limit may be in the other direction (MULLER 1926). The latest method (MULLER and PROKOFYEVA 1934), based on estimating the minimum number of different positions of breakage in a minute, circumscribed region of a chromosome, uses the genetic method referred to in the fourth preceding paragraph to define whether or not the breaks considered are in "sensibly the same" position. Since it suffers from the difficulty above explained, of failing to detect any genes (or gene-complexes or gene-parts) a deficiency of which would not result in lethality or readily detectable abnormality, it gives only a minimal number for the positions of breakage, and consequently for the number of genes, and a maximal gene size (though the former is considerably larger and the latter considerably smaller than any figure hitherto arrived at by other legitimate methods).

Although there is nowadays little practical disagreement among geneticists as to what constitute the criteria for distinguishing the gene material, or genetic material, from other material of the cell (the definition

involving the concept of self-determination of its own characteristics by the mother material in the reproduction of the daughter material, together with a mutability that does not interfere with this property), no such understanding has been arrived at concerning the question of how the limits of *a* gene, as distinguished from its neighbor genes, shall be defined. In genetic theory, genes have been considered as (1) crossover units—hypothetical segments within which crossing over does not occur; (2) breakage units—again hypothetical segments within which chromosome breakage and reattachment do not occur (at any rate, not without destruction of one or both fragments); (3) mutational and functional units —those minute regions of the chromosomes, changes within one part of which may be so connected with changes in the functioning of the rest of that region as to give rise to the phenomenon of (multiple) allelism; or (4) reproductive units—the smallest blocks into which, theoretically, the gene-string could be divided without loss of the power of self-reproduction of any part. A category of auto-attractive units might also be added.

Although it seems often to have been assumed, there is as yet no empiric evidence, and only doubtful theoretical ground, for assuming that the lines of demarcation between genes, as defined on any one of these systems, would coincide with those on any of the others, or even for assuming, in the case of some given one of these systems (especially the mutational one) that such lines of demarcation are necessarily invariable, non-overlapping, well defined and absolute. The minimal gene numbers and maximal gene sizes hitherto arrived at have to be considered in relation to the particular method used in arriving at them, one value having been a limit obtained through crossover studies, another through studies of mutation (allelism), and the most recent one through breakage studies. Hence they do not necessarily represent the same thing, even though it might be simplest to assume that they did.

In view of the above considerations, it is not far fetched to imagine that the "gene for scute," as recognized by the test of allelism of its mutations, may nevertheless consist of an undetermined number of parts, some of which may become separated from others by breakage without such separation resulting in the loss of the reproductive power of any of these parts (although, perhaps, interfering with their functioning). These parts, then, might themselves be denoted "genes," and the whole a "gene-complex," or the parts might be called "sub-genes" or something equivalent, and the whole a "gene," depending upon the taste of the writer and upon the criterion which he prefers (that of "allelism" or that of breakage) for defining the limits of a "gene."

In fact, in the case of achaete and scute, we do have a situation verging on that above imagined, inasmuch as, firstly (contrary to the earlier belief

of the workers on scute mutations), a recessive mutant gene, or a loss, at either one of these loci, does, although only in slight degree, act as an allele of such a condition at the other locus, both in respect to the achaete and to the scute characters, and, secondly, some "gene mutations," such as scute-3, affect both characters strongly, and act as simultaneous alleles of mutants of both types; yet both breakage tests (SEREBROVSKY and KAMSHILOV (unpubl.), and possible evidence by AGOL 1931) and crossover tests (DUBININ, SOKOLOV, and TINIAKOV, 1937) have shown the "genes" of achaete and scute to be separate in both the latter senses. Since we must thus admit that the original "scute gene," as defined by the mutational test, is really divisible into two parts, "achaete" and "scute" proper, as defined both by the tests of breakage and of crossing over, we should exercise caution before denying that the remaining "scute" proper may be further divisible, even though the parts of it might not have nearly as completely differentiated functions as the earlier postulated "sub-genes" were thought to have.

It was in fact in the hope of throwing light on the above question, more than on any other, that our series of studies on scute, of which the present one forms a part, were undertaken. Among the results of the series of studies was the finding that, of thirteen breaks at or near scute that were examined with reference to each other by the method previously referred to, of recombining the portions to the left and right of the breakage points, there were in all only four distinguishable positions of breakage—distinguishable by the criterion of having between them genes (or genetic material) the deficiency of which caused lethality or some readily distinguishable abnormality. Five of these breaks are to the left of scute: two of these—*y3P* and the left break of scute-19—to the left of both achaete and yellow; two others—*y4* and *y5*—to the left of achaete and possibly of yellow; and one—*sc-8*—between the genes achaete and scute. The three breaks here studied in detail (those of scutes 4, S1 and L8) appear to be immediately to the right of scute. And the remaining five breaks—those of scutes 7, 9, 29, and S2, and the right break of scute-19—are still farther to the right, as recombinants having the part of the X chromosome to the right of one of the latter breaks and that to the left of one of the three here studied were found to be lethal.

It hardly seems possible that it should be a coincidence that all three rearrangements having one breakage immediately to the right of scute, as in scute-4, should have had their other breakage in the "inert region" of the X, to which the scute region accordingly became attached, while all five having the breakage of the scute region located farther to the right (to the right also of the right-hand "lethal locus") should have had their scute region attached to some other, non-chromocentral, region. It seems

difficult to avoid the conclusion that other kinds of reattachments would have resulted in position effects that were either invisible or dominant lethal, and so escaped detection.

As cytological examinations of PROKOFYEVA have proved most clearly in the case of scute-19, all these breaks, and hence the genes between them, lie within a limited region of the second conspicuous band, or rather, band-complex, found in passing rightwards along the salivary X chromosome (in the region designated as 1 B 1 and 2 in BRIDGES (1938) diagrams). The most detailed studies have shown that not more than two entire bands, as seen with visible light, can lie between the leftmost and the rightmost of our breakage points (that is, between the left and right breakage points of scute-19), and the ultraviolet photographs of ELLENGORN (ELLENGORN, PROKOFYEVA and MULLER 1935) have shown not more than three bands. If we regard yellow and achaete as separate loci, and then take into consideration the evidence showing four different breakage points, we must conclude that there are at least four genes in this tiny region, hence more genes than detectable bands.

Is it however, legitimate to infer that there are only four genes, in the sense of independently reproducible segments of genetic material, separable by breakage, within this space? The finding that all the 13 breaks seemed to fall into just four positions instead of 13 would, to be sure, seem to show a considerable limitation in the number of possible breakage points here. But, when the method of determining whether or not two breakage points are in different positions is taken into consideration, it must be admitted that smaller differences in position might conceivably have escaped detection. For, in view of the fact that the deficiency of both yellow and achaete together was found not to be lethal, and that the deficiency of "scute" itself (that is, of the region between our second and third distinguishable positions of breakage) is not lethal in quite 100 percent of cases, it would be strange if yet smaller deficiencies were not more viable.

If these smaller deficiencies involve genes or "subgenes" that form part of the hypothesized scute complex and have their morphological expression mainly in bristle pattern, it might be thought that it should have been possible to detect them by reason of the relative absence of bristles in one of the classes of recombinants (the deficient class) as compared with the other classes. Yet, as we have seen, there was no evidence of such complementary action. There are, however, several circumstances that might well interfere with the detection of such subsidiary parts by this means. In the first place it might be that portions of the complex, when removed from some chief grouping, came, through a species of position effect, to have little or no influence upon the character in question (here, bristle frequen-

cy); thus the really deficient class might show little or no more evidence of the deficiency than one of the original classes, in which the parts were all present, but merely separated. Secondly, we know, as a matter of fact, that these rearrangements have somehow brought about a drastic reduction of effectiveness (in bristle production) of the scute gene or scute complex taken as a whole, and in this state of very low bristle frequency (involving the total absence of many bristles ordinarily very responsive to variable influences), slighter influences, due to the presence or absence of some weakly acting minor part of the whole, might well escape detection. Under these circumstances, even quantitative phenotypic studies on truly isogenic material might fail to give evidence of the gene complex having been divided. The locus of yellow affords an even better illustration of this principle than does that of scute, for in the presence of the inversions called yellow 4 and 5 the character-effect of the locus is completely absent (the flies being completely yellow, as when the gene itself is absent); and so, far from telling (by our method of the recombination of rearrangements) whether the breaks in question may lie within some yellow gene-complex, it is impossible even to know whether they are just to the right or just to the left of it, or whether, perhaps, a break had occurred on both sides simultaneously, with resultant actual loss of the gene.

In the case of the scute rearrangements, we have seen that, although the apparent differences in effects of the right ends of the inversions do at first sight suggest that they contain parts of a scute complex, which had been transplanted to these positions by the process of breakage and reattachment, nevertheless the irregularity of the same effects, and the lack of any tendency for the effects of the right and left ends to be complementary, destroy the usefulness of such an interpretation of them and make it more likely that these differences have had no connection with the nature of the rearrangements. On the other hand, the rather regular differences shown by the left ends can, *a priori*, be interpreted equally well as due to differences of position effect caused by the presence or absence of bobbed, block *A*, and/or another, invisible gene, or genes, from either the right or left ends or both, that have in the main other functions than those involved in the "scute" character itself; or as due to the separation from the scute gene or gene-complex of relatively minor parts of it which function little or not at all when separated from the rest of it, but which when in the neighborhood of the latter, through their interaction with it result in a considerable increase of bristle production, involving different bristles to differing degrees. It should be observed that these two hypotheses really run into one another, for by admitting, on the former hypothesis, more and more independently reproducible genes or gene-parts, derived from the left end, and by more and more reducing their action, when in their orig-

inal position, to that concerned with the pattern of the bristles, we gradually arrive at the second hypothesis.

The real question here, then, is to what extent there may exist, in the scute region of the normal X chromosome, a series of elements that can be separated from one another, without losing their capability of reproduction, and that, at least when in their normal positions, co-operate in the production of the given character (the bristles produced by the normal allele of the scutes). Still another question is that of the possible existence of elements, not capable of reproduction except in their original setting, that are subject to being cut off from their reproductive base by chromosome breakage. It may also be questioned to what extent the functions of these elements differ from one another (as, for instance, to what extent they may affect different bristles), and to what extent each of them may subserve other, differing characters as well. Certainly the "gene" for achaete, just to the left of scute, has some part in this scute complex. And our series of studies on the chromosomal conformation of the other alleles shows that, just to the right of the main scute locus also, there lie one or more genes which (as compared with genes that may be substituted for them by inversion or translocation of other euchromatic regions) exert an appreciable position effect assisting in the production of the normal ("non-scute") bristle characters. At least one of these genes is not immediately adjoining the scute locus but lies to the right of the locus of the lethal which itself is to the right of scute, because the scute character is affected even when the chromosome is broken and rearranged to the right of this lethal locus. We cannot, at present, be sure that between the boundaries so set, or even within the main "scute locus" itself, the genetic material is not still further divisible, in the sense defined, nor know to what an extent it may be thus divisible. Certainly neither our experiments here presented, nor our whole series of studies on scute, give good evidence for the existence of such further divisibility, but it is equally important to note that they do not give convincing evidence against it either, as they might seem to do on first consideration, and, in fact, it is quite possible that a part of the effects here noted are results of such divisibility.

We have laid emphasis on this matter because this question of the further divisibility (without loss of reproductive capacity of the elements) of regions of the chromosome that have, on other grounds, been considered as probably representing single "genes," has recently been brought to the fore by various results in the field of gene mutation and chromosome rearrangement. In the first report of the finding of minute rearrangements, and of the phenotypic changes accompanying them (MULLER, PROKOFYEVA and RAFFEL, 1935), the suggestion was made that many, perhaps all, of the supposed gene mutations produced by irradiation, and probably some

at least of the spontaneous ones as well, represented fundamentally the same phenomenon, although on an even smaller scale than that found in our cytologically demonstrable cases. But at that time we did not believe that gene mutations in general—especially those forming the basis of evolutionary progression—could be of such type, because we did not think of "genes" (as defined by the mutational properties of a given chromosome region) as being further genetically divisible, and we realized that the number of kinds of somatic changes dependent upon the limited position effects of rearranging these relatively large and few units could not be nearly of the order of magnitude necessary to provide for the great evolutionary plasticity and diversity of organisms. Since then, however, considerations of the similarity in expression, and in mode of production, of "gene mutations" and of minute rearrangements, and of the empirical impossibility of drawing a line between them, have led us to question whether or not there may be even minuter rearrangements than those which change the position of "whole genes" (as taken in the older, less flexible sense). For if smaller, linearly arranged parts of those regions which have been considered as genes (by criteria of mutation and allelism) may become rearranged with respect to each other in essentially the same manner as the "whole genes," then, at least by having such parts rather numerous and of a number of diverse kinds, sufficient plasticity of result is introduced to make plausible the conception that most gene mutations might be of this kind.

Among the findings which played an important part in leading us to put the present question, was that concerning the effect of varying the dosage of radiation upon the production of mutations. It had been found by MULLER and various co-workers (KOERNER, VOGT, BELGOVSKY, BERG, PANSHIN and BORISOFF), and later confirmed by certain results of DUBININ and his colleagues, that the frequency of rearrangements that are readily detectable as such genetically, that is, "large rearrangements" induced by X rays, is proportional to more than the first power of the dosage (ionizing capacity) of the rays—the value being, for the doses ordinarily used, about half-way between the first and second power. On the other hand, as various workers have shown (OLIVER, HANSON, TIMOFÉEFF-RESSOVSKY and others), and as special studies of RAFFEL have recently confirmed, the frequency of the "gene mutations" is simply proportional to the first power of the dosage of radiation. Here then there seemed to be a rather fundamental distinction between the mechanism of production of rearrangements, which presumably always involve at least two breaks and so may depend more nearly on the second power of the number of ions, and "gene mutations," which conceivably involved (at least in their initial stages) but one chemical substitution.

The possibility had not yet been excluded, however, that *minute* rearrangements might obey the same dosage rule as gene mutations, for they had not yet been tested in relation to this question, on account of the difficulty usually encountered in obtaining enough recognizable cases of them. Studies of BELGOVSKY, PROKOFYEVA, RAFFEL and MULLER, however, showed that, by the use of special stocks, minute rearrangements in or near certain chromocentral regions could be obtained and recognized in sufficient quantities, and it was accordingly decided that the frequency of the production of these rearrangements by different doses of X-rays should be measured. This has been done both by BELGOVSKY and by MULLER and MAKKI (see BELGOVSKY 1939, MULLER, MAKKI and SIDKY 1939, MULLER 1939) and their results have shown clearly that these minute rearrangements vary as the first power of the dosage, just like the supposed gene mutations. This result now breaks down the last empirical distinction that it was possible to draw between "gene mutations" and "chromosome rearrangements" and opens wide the door for considering the former also to result from double or multiple breaks, with reattachment, like the latter, but on an even minuter scale. And this in turn raises questions concerning the limits of what have been called "genes," and the propriety of assuming that "genes" as conceived according to our different hypothetical criteria are necessarily coextensive.

Since the above possibilities were suggested by MULLER (1937) some similar suggestions have independently been made by GOLDSCHMIDT (1937 a, b, 1938). Although we differ from GOLDSCHMIDT in that we regard our suggestions as pointing, as yet, only to possibilities, while he regards his corresponding proposals as representing something highly probable, and although we differ also in various matters of emphasis, implication, and subsidiary interpretations, as well as in what we regard as the more valid grounds for regarding such a conception as plausible, we must nevertheless agree in the more general proposition, central to his theme and ours, that the "gene," in the rather loose sense in which it has so long been taken for granted by most geneticists, may *perhaps* be genetically further divisible, even into many genetically linearly arranged portions of semi-autonomous character (autonomous in the sense of their being able to reproduce when in other linear arrangements), and that, contrariwise, neighboring "genes" often or usually co-act, in a manner made possible by their juxtaposition, so as to produce character-effects that depend upon overlapping regions, somewhat larger than a single "whole gene," as formerly conceived. The idea of a "whole gene" or, more directly, of "a gene" itself, thus comes under scrutiny, and we are at present far from having evidence that the "whole gene" or "the gene" (meaning just "*one* gene") would be the same when defined by the various different criteria listed. We should not wish,

however, to deny the alternative possibility, that the in some ways simpler-seeming conception of indivisible genes in the older sense (modified, however, by the conception of the position effect) may eventually be found to correspond to the facts, after all. What we do wish to emphasize chiefly is that more work will be needed before the question can be decided.

Whether genetics and cytology themselves will provide methods adequate for solving such remote questions it is as yet too early to say. As an example, however, of a type of genetic study that might conceivably throw light on a question of this kind, we shall mention a study undertaken by MULLER, with the aid of SINITSKAYA, to determine whether it was possible to induce reverse mutations in scutes of the type reported upon in the present paper. If these scute mutations represented position effects of the rearrangements of "whole genes," in the older sense, then it should much more readily be possible to obtain reverse rearrangements that would restore the original order, and the normal phenotype, than if the scute mutations represented rearrangements the breakages of which had come between smaller parts of the "gene." For in the latter case new breaks would much more rarely (especially when both of them were considered) come between just the same two parts as before—a condition that might well be necessary in the case of so manifestly complex a gene as that for scute. In the latter case, too, there would have been a chance that a part of the gene had been lost when the original mutation occurred, and so could not be restored. On the other hand, in case "whole genes" were concerned, there would be especial reason for hoping that exactly reverse rearrangement might be found within a reasonable time, because it is known that breaks occur much oftener in or near the chromocentral region, and the scute rearrangements here in question are, as we have seen, such that both points of reattachment now adjoin such a region.

In order to increase the chance of breakages occurring near both of the original positions at once, a recombination chromosome was used which had the right end of scute-4, which contains the longest chromocentral region to the right of its right break, and the left end of scute-L8, which contains more chromocentral region just to the right of its left-hand break than scute-4 does, since bobbed is included in this region in scute-L8 but not in scute-4. (Unfortunately at that time it was not yet known that block-A was not in this region of scute L8, along with bobbed, otherwise the left end of scute-S1, which has both these genes at the left end, would have been used.) Scute males having the above recombination chromosome were then X-rayed with a dose of about 3,000 *r*, and crossed to females with attached X chromosomes, and their sons were examined for possible reverse mutations of scute to a normal or near-normal phenotype. How-

ever, among the approximately 50,000 male offspring examined, none showed such a change.

We can by no means regard this negative result as being in itself of much significance, until the frequency with which breaks in and near such chromocentral regions occur becomes better known, and it will be desirable to have the experiment repeated, with even larger numbers. We present it only to show that the possibilities of investigating the general problems here at issue by means of genetic tests are as yet by no means exhausted.

All in all, we do not wish to give the impression that our results with the three scutes here reported upon actually support the idea of a finely divisible gene. They do, however, give evidence for the position effect, and this phenomenon in itself connotes that the gene, as defined by the mutation-allelism test, extends over a larger region than that defined by breakage, crossing over or self-reproducibility, and that, in fact, the regions of successive genes, as defined by the mutation-allelism test, do not merely adjoin but overlap each other. On the other hand, our present results do not, in themselves, give evidence of a finer divisibility than that found in our more general study of breakage in the scute region, which had indicated the existence of at least four separable genetic constituents ("genes") within the space of one or two visible bands, and which further showed that, if other breakage points than those forming these four parts existed, the elements included between them must be undetectable by ordinary genetic tests. It was, then, not these results, but those of other investigations, especially the recent ones on minute rearrangements and their relation to dosage, those on the phenotypic relations of achaete and scute, and general considerations based on the manner of production of mutations and the nature of the gene, that made it necessary to take up in some detail here the question of finer gene divisibility, and to consider to what extent our present results might be in favor of or opposed to such a conception. As we have seen, they can be interpreted in either way with roughly equal plausibility (aside from extraneous considerations that might affect the plausibility of the divisibility idea). Hence, so far as our present results are concerned, this matter must still be left an open question.

It should eventually be possible to obtain further light on this question through the obtaining of additional scute alleles and their intensive study. The next time an inversion occurs with the left break in the same apparent position as that of scutes 4, S1 and L8, and the right break just to the right or left of *bb* and block A or between them, will it, when made isogenic with whichever of these three scutes whose arrangement it seems to duplicate, also be identical with that scute phenotypically? If so, it is unlikely that the "scute gene" is subdivisible into different parts affecting the bristles. Such work is more or less complementary to the study of the effects of re-

verse rearrangement above discussed. And, in general, a continuance of intensive work on breakage in the scute region should give more information about the number of possible points of breakage.

There are other considerations bearing on the question of the segmentalism of the genetic material, for which the reader may be referred to another paper (MULLER 1940). Among these are the results, obtained by MULLER and MACKENZIE (1939) since the present paper was written, showing that ultra-violet light produces gene mutations but not gross rearrangements. This and other facts would argue for a more fundamental distinction between gene mutations and rearrangements than that above suggested as one of the alternative extremes.

SUMMARY

1. Analysis of the three scute mutations, scute-4, scute-S1 and scute-L8, produced by X-rays, shows that all three involve an inversion, having its left break close to the right of the "scute gene," and its right break in the proximal chromocentral ("inert") region of the X chromosome. None of the numerous other scute mutations investigated has breaks in both of these positions.

2. Single crossovers of both contrary classes between each of these inversions and each of the others are viable, and phenotypically similar to the original types (except as noted in section 5, following). Hence all three of the left hand breaks must have been in identical positions or else in positions so close to one another that deficiency of the region between them was neither lethal nor productive of any detected abnormality (except as noted in section 5). Salivary study by PROKOFYEVA revealed no difference in positions of the left-hand breaks.

3. Genetic testing of the crossover chromosomes for bobbed and cytological testing (in metaphase stage) for block A showed that, while in scute 4 the right-hand break was to the left of both these genes, in scute-S1 it was to the right of both of them, and in scute-L8 between them, carrying bobbed to the left end and leaving block A at the right, a result that had probably been made possible by the occurrence of a further, very minute, rearrangement, whereby these two genes interchanged their positions. If other genes differentiated the positions of the right-hand breakage points, the absence of these from the X chromosome produces no effect detectable by the methods used. Salivary study by PROKOFYEVA showed that the right-hand breaks were differentiated by only one or two faint lines of the chromocentral region.

4. Phenotypically, these three mutations, and also the recombinations between them, resemble each other, but the exact extent of the resemblance or difference could not be gauged until isogenic groups of all nine

of them (the three original combinations and the six possible recombinations between them) were constructed. The methods are given whereby, through a scheme of crosses involving 26 successive generations, the nine lines isogenic for all of the X chromosomes except their very ends were constructed, and whereby, through ten further generations, these nine groups were made isogenic for their major autosomes (again except for their very end regions) at the same time.

5. One of the recombinations between the original scutes (that having the left end of scute 4 and the right end of scute-L8) proved to be sterile, at least in the male, but when the isogenic lines were obtained the same type of recombinant, having the bulk of its X chromosome replaced by that of the standard X used, was as fertile as the other scutes. This shows that complementary genes for sterility had arisen in the X chromosomes of the two scute mutants, lying in different loci, and (at least one of them) separable from and not dependent upon the points of rearrangement of the inversions. Alone, neither caused sterility, but only in combination with one another. This serves to illustrate the mode of origination of genetic isolation in the evolutionary splitting of species.

6. Comparison of the nine isogenic groups shows them all to be much more similar to one another than any of them was to any of the numerous other investigated scute mutations. This, taken in connection with the unusual similarity of all of them to each other in gene arrangement, proves that there is a close connection between type of gene arrangement, that is, between the kind of neighbor genes a given gene possesses, and type of phenotypic effect, that is, the phenotypic similarity must be due to the "position effect" of the genes, and their change of expression, seen as the visible scute mutations, must have been due to the change of gene positions relative to their neighbor genes.

7. Despite the great similarity, there were unmistakable differences between the different types which it is logical to refer to the relatively slight differences in their gene arrangement.

8. Analytical comparisons of the nine classes and of averages between certain groups of them, showed that the left ends of the inversions differed in their effects in a consistent fashion, the order of abundance of bristles of practically all differentially affected types, in the presence of the left ends of the different scutes (with the right end derived from the same scute in all three cases), being scute-4 > S1 > L8. The isogenic lines of the original combinations of left and right ends showed the same order of effects. It is inferred that these differences in the left ends, and in the original scutes as a whole, are caused by the position effects, in connection with the scute gene, of genes that differentiate the present left ends of the chromosomes from one another, and that lie just to the right of the leftmost of these

scute breaks. Among these genes may be bobbed and block A, and possibly other "invisible genes" from the original right or left ends, or from both.

9. Although there were also some differences of statistical significance produced by the different right ends (in the presence of a constant left end), these were considerably less than the differences produced by the left ends, were not in the same direction in the case of different bristles, showed no consistency when the results from the different right ends in the presence of one left end were compared with the corresponding results in the presence of another left end, and showed no tendency to be complementary in value to the results from the left ends (as they should if due to a transfer, by the inversion, of different bristle-producing genes, or subgenes, from the original left to the right end). The interpretation accordingly suggests itself that the apparent differences obtained from the right ends might perhaps be really due to a lack of complete isogenicity on the part of the lines studied.

10. The question is raised of to what extent the breakages in the region of scute in this and other cases where they seem identical in position are really identical, or may be separated by parts of a "gene-complex" (or "gene," according to definition) which, although able to reproduce separately, function to produce bristles only, or mainly, when in proximity to one another, and function normally only when in proper arrangement. On account of the extreme reduction of bristles in these cases of nearly identical rearrangement, caused by the position effect, the influence upon the bristles of differences in such hypothetical parts might not be noticeable under these conditions. However, it is conceivable that they, rather than bobbed, block A, or other genes from the right end, are the chief differentiating factors that, by their differing position-effects with the rest of the scute complex, cause the differences that have been noted between the effects of the different left ends. Similar considerations apply to other loci, and make it uncertain to what extent our criteria as to what constitutes a single gene, based upon different methods of approach (herein listed), refer to co-extensive structures. These considerations likewise affect our judgments regarding gene size and number. The undoubted overlapping of function of portions of the chromonema distant enough to be separable by crossing over or breakage, caused by the position effect, raises further difficulties in the delimitation or definition of *a* gene. In recent publications GOLDSCHMIDT has independently expressed a point of view which is in some essential respects similar to this.

11. The finding of minute rearrangements, with position effects resembling gene mutations, has made the above question more acute, for on the hypothesis of independently reproducing gene-parts or "sub-genes" (or

genes within a larger gene-complex), the "gene mutations" *might* simply be rearrangements so small as to lie within a "gene" (or gene-complex). This would agree with the recent discovery by BELGOVSKY and MULLER that demonstrable rearrangements of minute size vary in frequency as the first power of the X-ray dosage, like "gene mutations," and differently from large rearrangements, the variation of which is, for ordinary doses, approximately as the $3/2$ power of the latter. However, the question of gene delimitation is as yet far from settled, being as yet in the earliest stage—that of being formulated. Certain genetic avenues of approach to it can be descried, of which one illustration is cited, which involved an experiment, having negative results, designed to find reverse mutations of one of the recombinational scutes here studied. The failure here would argue for a relatively high divisibility of the genetic material, but is not yet to be taken as more than merely suggestive, especially since other considerations and results point in the opposite direction.

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THE ACTION OF CERTAIN MUTANTS UPON THE PENETRANCE OF HETEROZYGOUS VESTIGIAL WING IN DROSOPHILA MELANOGASTER

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INTRODUCTION

ONE of the methods used in the study of the mechanism of gene action in *Drosophila melanogaster* has been to observe the modification of the phenotypic effects of mutant genes following alterations in the environment in which development occurs. Thus the effects upon the mutant phenotype of temperature changes, crowding of the cultures, and starvation of the developing individuals have been observed.

More recently, certain genetic and environmental factors which alter the duration and rate of development have been studied for their effects upon the mutant phenotypes. It has been found that the facet number in homozygous vestigial, Bar individuals is significantly smaller than in non-vestigial, Bar individuals (MARGOLIS 1935). Conversely, increase in the facet number of Bar individuals resulted when mutant larvae were starved (BODENSTEIN 1939). A decrease in the size of Lobe eyes occurred when certain of the dominant Minutes were introduced (DUNN and COYNE 1935). A systematic study of a number of eye and wing mutants showed an increase in the penetrance of all mutants studied when either of two dominant Minutes was introduced (BRYSON 1940). An increase in the penetrance of vestigial wings in the presence of dominigenes has been reported in flies heterozygous for vestigial and having also certain chromosomal aberrations (GOLDSCHMIDT, GARDINER, and KODANI 1939). By starving homozygous vestigial individuals, CHILD (1939) was able to observe an increase in the size of the imaginal wing. A similar effect for vestigial^{no} and cut^{n} has also been observed by BRAUN (1939).

The data presented here are the results of a study of the influence of two Minute mutations and of a duplication of the third chromosome upon the penetrance of the heterozygous vestigial wing in *Drosophila melanogaster*.

MATERIALS AND METHODS

The two dominant Minutes used here were respectively $M(3)w$ located at 80 on chromosome III, and $M(2)l^2$ located at 101 on chromosome II. Both cause a prolongation of the larval period. In the case of $M(2)l^2$ the mean prolongation of the larval period at a temperature of 25°C amounts to 12 hours (DUNN and MOSSIGE 1937). Among $M(3)w$ males the prolongation of the larval life is 41.3 hours, while among the females of this geno-

type the prolongation amounts to 42.9 hours at a temperature of 25°C. (BREHME 1939).

Duplication *T1305* females occur with a relatively high frequency (about 40 percent) when males heterozygous for *T1305*, a translocation between chromosomes I and III and marked with the dominant Dichaete, are mated to females free of the translocation. The resulting non-Dichaete females possess a duplication of an inner fragment of the right arm of chromosome III. Phenotypically, the duplication in the female produces a slight spreading of the wings, increased pigmentation of the abdomen, and, occasionally, rough eyes. Although two percent of the duplication males have nicked wings, no nicks were observed among three hundred females examined by OLIVER (1937). Unpublished data indicate that occasionally duplication females have nicked wings.

In all matings the female parents were homozygous for vestigial and were derived from a vestigial stock which had been inbred in mass culture for a period of two years. Females were mated to males $M(2)l^2$ balanced with *Cy*, or to males $M(3)w$ balanced with $In(3R)C$, or to males *T1305D*. As a control test, vestigial females were mated to wild-type males. In order to minimize any effect due to physiological differences in age, all females were selected as virgins and held for 48–72 hours. They were then mated and held in vials for 48 hours, and then transferred without etherization to half-pint milk bottles. Each culture contained approximately 50 cc of the regular banana-agar medium, enriched with brewers' yeast, and seeded with two drops of a thick yeast suspension. In order to minimize the factor of crowding, five or six pairs of parents were used to a mating, and females were allowed to oviposit only for a period of twelve hours before they were transferred to fresh bottles. The total time of oviposition of any female never exceeded sixty hours. All experiments were conducted at a temperature of 23 ± 1°C.

EXPERIMENTAL

Prolongation of the developmental period

No specific study was made upon the prolongation of the various stages of the life cycle; but in the course of the experiment, observations were made upon the length of the total life cycle of the $vg/M(2)l^2$ and $vg/+$, $M(3)w/+$ individuals. The delay as based upon the time of emergence of these individuals at 23° is in agreement with that expected on the basis of the cited experiments. It was found that the $vg/M(2)l^2$ individuals emerged approximately 18 hours later than the $vg/+$ individuals, and that the $vg/+$, $M(3)w/+$ individuals emerged approximately 60 hours later than the non-Minute sibs. These measurements are based on twelve hour egg-laying periods, and observations of emergence every twelve hours. In all

cases the males emerged later than the females. No precise measurements of the prolongation of the developmental period in duplication *T1305* were made; however, it was noted in series tested concurrently that the time of emergence was later than *vg*/+ males but earlier than the emergence of the *vg*/*M*(2)*l*^{2} females. On the basis of these observations the compound genotypes considered here are seriated in order of increased length of developmental periods as follows: *vg*/+ ♀ ♀; *vg*/+ ♂ ♂; *vg*/+, duplication *T1305* ♀ ♀; *vg*/*M*(2)*l*^{2} ♀ ♀; *vg*/*M*(2)*l*^{2} ♂ ♂; *vg*/+, *M*(3)*w*/+ ♀ ♀; and *vg*/+, *M*(3)*w*/+ ♂ ♂.

Effects on the heterozygous vestigial wing

From the data obtained it is clearly evident that the introduction of either *M*(2)*l*^{2}, *M*(3)*w*, or the duplication *T1305* into heterozygous vestigial individuals resulted in a modification of the wing phenotype, and that the modification is expressed in the form of notching and nicking of the wings. In each case, the frequency of wing scalloping is significantly greater than in the controls.

TABLE 1

Effect of three genetic factors on the heterozygous vg phenotype. Frequencies in percent with standard error for each of three phenotypic classes. Genotypes listed in order of increased length of developmental period.

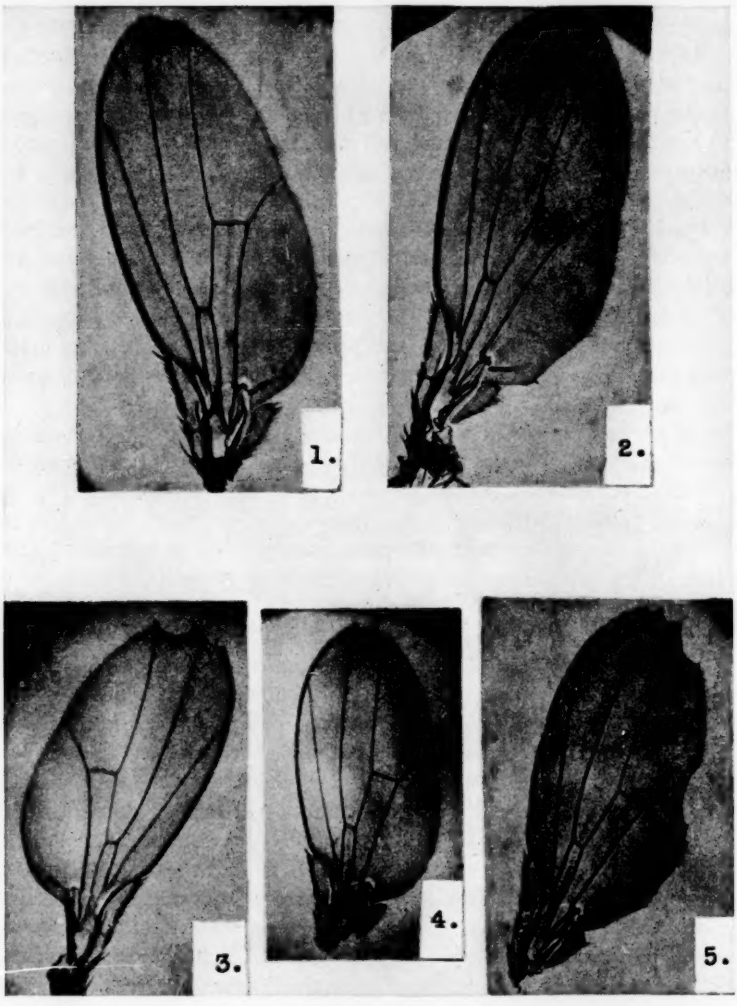
GENOTYPE	PERCENT WINGS+	PERCENT 1 WING SCALLOPED	PERCENT 2 WINGS SCALLOPED	TOTAL PERCENT SCALLOPED	N
♀ *vg*/+	100.00	0	0	0	257
♂ *vg*/+	99.60±1.27	0.40±1.27	0	0.40	248
♀ *vg*/+, *T1305D*$^+$	67.09±1.77	29.33±1.72	3.58±0.70	32.91	699
♀ *vg*/*M*(2)*l*^{2}	86.85±1.23	13.01±1.23	0.13±0.13	13.14	753
♂ *vg*/*M*(2)*l*^{2}	62.28±1.79	30.28±1.74	7.43±0.99	37.71	700
♀ *vg*/+, *M*(3)*w*/+	44.44±1.99	41.06±1.97	14.49±1.41	55.55	621
♂ *vg*/+, *M*(3)*w*/+	9.86±1.16	34.14±1.84	56.00±1.93	90.14	659

In table 1 the genotypes are arranged in sequence from the shortest to the longest length of life cycle. From the table it is apparent that the control counts are smaller than the counts in the several test groups. However they are in agreement with the control counts reported in our preliminary account (GREEN and OLIVER 1940) in which it was found that among the females *vg*/+ the frequency of wing scalloping was 0/365, while in the males *vg*/+ the frequency was 3/315. The preliminary data for control tests as well as those for the two Minutes were not included in this table because different culture media were used. The media of the earlier tests lacked brewers' yeast.

With each genotype tested, it is noteworthy that the males in addition to having a slightly longer developmental time also showed a greater frequency of wing scalloping than the females. An association between the developmental time and the frequency of wing scalloping is also observed with the various genotypes. On the basis of total wing scalloping (that is, the sum total of the flies with one wing incised and with two wings incised), the increase in scalloping is in accord with the increase in developmental time for a given genotype, with the one exception of the duplication *T1305* females. Although the prolongation of the life cycle was less in the *vg*/+, duplication *T1305* females than in the *vg*/*M*(2)*l*^{2} females, the frequency of wing scalloping in the duplication females, 33 percent, more nearly approached that of the *vg*/*M*(2)*l*^{2} males, 38 percent, and was greater than the frequency observed in the *vg*/*M*(2)*l*^{2} females, 13 percent.

If the frequency of two wings scalloped is taken as a measure of the penetrance, it is apparent that the penetrance increased in the same order as the total wing scalloping frequency. In the controls no case of two wings scalloped was observed. This was true also of the controls of our earlier account (GREEN and OLIVER 1940). In the genotypes containing either the Minutes or the duplication, the least penetrance was observed in the *vg*/*M*(2)*l*^{2} females, with only 1/753 individuals exhibiting two scalloped wings. The penetrance increased progressively with an increase in the length of the developmental period, until among the *vg*/+, *M*(3)*w*/+ males, 369/659 individuals possessed two scalloped wings. Again the data on the duplication females fail to fit the general results.

Because of the variability in the degree and region of the wing scalloping, no quantitative measurements of the reduction in wing size were attempted. However, certain variations in the general degree and region of the wing scalloping were noted among the genotypes tested. Among the *vg*/*M*(2)*l*^{2} females, with the exception of one case, only one wing was affected. Generally, the nick or notch occurred in the inner margin of the wing in the region of the third posterior cell (figure 2). Occasionally the apical region, or the outer margin was affected. Among the *vg*/*M*(2)*l*^{2} males, the frequency of two wings scalloped increased to approximately one-fifth of the total scalloped individuals. The degree of manifestation increased only slightly above that of the females of this combination. Generally, the apical region of the wing was nicked or notched (figure 3). Occasionally the inner margin was notched. The frequency of one wing scalloped among the *vg*/+, duplication *T1305* females was approximately equal to the frequency of this class among the *vg*/*M*(2)*l*^{2} males. However, the frequency of two wings scalloped in the duplication females was considerably less than in the *M*(2)*l*^{2} males and composed only about one-eighth of the total scalloping frequency of these females. With but few exceptions the nicking



FIGURES 1–5.—Alterations of the phenotype of *vg*/+wings. Figure 1. normal type of wing, *vg*/+female. ×30. Figure 2. wing with inner margin incised, *vg*/*M*(2)*l*^{2} female. ×30. Figure 3. wing with apical notch, *vg*/*M*(2)*l*^{2} male. ×25. Figure 4. wing with nicks in apex and inner margin, *vg*/+, *M*(3)*w*/+female. ×20. Figure 5. wing with extreme scalloping, *vg*/+, *M*(3)*w*/+male. ×25.

of the wings was confined to the apical region of the wing. Occasionally the wing was notched. Approximately one-fourth of the *vg*/+, *M*(3)*w*/+ females with scalloped wings had both wings scalloped. Either the inner margin of the wing was incised, or the apical region was nicked or notched. On a number of occasions both of the regions were affected in the same wing (figure 4). The most extreme manifestation occurred with the *vg*/+, *M*(3)*w*/+ males where almost two-thirds of the scalloped individuals had both wings scalloped. In almost every case the apical region of the wing was clearly notched, and in many cases the inner margin of the wing was incised at the same time (figure 5). In a few cases the inner and outer margins as well as the apex were incised, giving the wing the appearance of the Beadex phenotype.

DISCUSSION AND CONCLUSIONS

In the data presented it has been pointed out that a definite modification of the heterozygous vestigial wing phenotype results when either of two Minutes, *M*(2)*l*^{2} and *M*(3)*w*, or a duplication of chromosome III, duplication *T1305*, is introduced.

The action of the Minutes on vestigial is probably general in nature, and associated in some manner with the prolongation of the developmental time. The Minutes are known to have a modifying effect upon various genes (DUNN and COYNE 1935; BRYSON 1940) other than vestigial. They do not cause scalloping of the wings; and therefore the increased frequency of scalloping in the combination of Minute with *vg*/+ is not likely to be an expression of the added individual actions of the two genotypes. However, duplication *T1305* may in part involve exaggeration, as will be discussed later. Although the Minutes cause a decrease in total growth (BREHME 1939), it does not seem possible to explain the scalloping on that basis. A decrease in body size occurs as a result of starvation of homozygous vestigial flies (CHILD 1939) and of crowding of Minute, *vg*/+ individuals (unpublished data); yet an increase in wing area occurs. If the general growth rate is a factor in scalloping, a further decrease in wing area would be expected with the smaller individuals.

Association with prolonged development

Each modifier, *M*(2)*l*^{2}, *M*(3)*w*, and duplication *T1305*, when introduced into *vg*/+ caused an increase in the length of the developmental time for the individuals as well as an increased frequency of wing scalloping. The similar direction of the prolongation of development and of the frequency of scalloping indicates that in some manner the two phenomena are associated in a causal way. With one exception, the genotype which caused an increase in the length of the life cycle caused a like increase in the frequency

of scalloping as measured by the total number of flies with scalloped wings. Similarly, the increased life cycle was associated with an increased degree of scalloping, as measured both by the quantity of wing present and by the number of flies with both wings incised. Individuals with the $M(3)w$ combination have a life cycle considerably longer than those with the $M(2)l^2$ combination, and the frequency and degree of scalloping are greater in the former than in the latter. Males have a longer life cycle than females; and with each combination, males have a higher frequency and to a lesser extent a greater degree of wing scalloping. However, the observed frequency of scalloping in the males exceeds the frequency expected if the effect is strictly proportional to the prolongation of the developmental time. A similar sexually dimorphic effect has been noted in temperature studies (HARNLY 1930; STANLEY 1931) and in starvation experiments (CHILD 1939) made upon homozygous vestigial.

The only discrepancy in the close similarity between the frequency of scalloping and the prolongation of development occurred with duplication *T1305*. On the basis of total length of life cycle, the frequency of scalloping in $vg/+$, *T1305* duplication females should be less than that observed in $vg/M(2)l^2$ females. The observed frequency, only slightly less than that for $vg/M(2)l^2$ males, was greater than expected. However, the prolongation associated with the Minutes occurs during the larval period (DUNN and MOSSIGE 1937; BREHME 1939). In our experiments, the length of life cycle for duplication *T1305*, as well as the Minutes, is a measure of the total life cycle. The exact stage associated with prolongation in the duplication is unknown. A second factor involved in the discrepancy may be the appearance of nicked wings in duplication individuals (2 percent in males) and in $vg/+$ males (0.4 percent). Since each genotype tends to produce scalloping, the combination $vg/+$, *T1305* duplication in the females might be expected to exaggerate the frequency of scalloping. It is known that the duplication in combination with Notch greatly exaggerates the Notch character (OLIVER 1937).

Although prolongation is apparently an important factor, the exact method by which it acts to increase the frequency and penetrance of vestigial is problematical. The gene for vestigial seems to be most susceptible to developmental modifications occurring during the larval period, and the Minutes prolong the larval period. Evidences for the larval susceptibility of the combination Minute, $vg/+$ have been shown by the response of the combination to temperature changes which were altered only during the larval period (GREEN and OLIVER 1940). The action of the prolongation upon the phenotype may be associated with the nature of the gene involved.

The gene for vestigial has been subjected to several studies in an at-

tempt to learn its nature of action. The data which we have presented do not clarify the picture but certain conditions are suggested.

If the gene for vestigial acts to produce a growth substance (GOLDSCHMIDT 1938) but does not produce enough of that substance for normal wings to differentiate, then our data on prolongation do not fit clearly into the hypothesis. Prolongation of development with the genetic combinations studied is associated with a decrease in wing area; and there is a definite correlation between length of the developmental period and the increased frequency of wing scalloping. The opposite effect should be expected if more time is allowed for vestigial to produce more growth substance.

If, however, the gene for vestigial acts to produce something which either inhibits or destroys a wing substance already present, prolongation as such might be a factor in scalloping. Delay in development during the sensitive period can permit the inhibiting substance to act for a greater interval of time, or permit the production of a greater amount of the substance. As a consequence, the wing which began as a normal structure would fail to develop completely. That type of action has been reported in a study of the pupal wings (GOLDSCHMIDT 1937), but no study of a similar nature has been made on the larval wing buds. That an inhibitory factor may be active during the larval period is suggested from the observations of CHEN (1929) who found that the wing anlage of wild-type larvae at pupation are larger than the vestigial anlage at the same period.

SUMMARY

1. A study of the effects of three genetic factors, *M(2)l²*, *M(3)w*, and duplication *T1305*, all of which prolong developmental time, upon the wing phenotype in heterozygous vestigial was made.

2. It was found that in all cases studied the wing phenotype was modified in the form of notching and nicking of the wings. In each case the addition of the Minutes or the duplication significantly increased the frequency of wing scalloping over that of the controls.

3. The frequency and degree of wing scalloping were found to be associated with, but not proportional to the extent of prolongation of the developmental period by the Minutes; the greater the prolongation, the greater the frequency and penetrance of the wing scalloping. A sexually dimorphic effect was noted.

4. The frequency of wing scalloping which resulted with the introduction of the duplication was greater than that expected on the basis of prolongation of the developmental time alone.

5. It is suggested that the action of the Minutes is related to their effect on prolonging the action of the vestigial gene at a specific time during de-

velopment. It is also suggested that the action of the duplication is related to the prolongation of developmental time, as well as to the effect of the duplication alone on the wings.

6. The suggestion is offered that under the conditions of these experiments the gene for vestigial acts to elaborate some wing inhibitory or destructive substance.

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SOMATOPLASTIC STERILITY AS A CAUSE OF SEED FAILURE AFTER INTERSPECIFIC HYBRIDIZATION

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COLLAPSE of the immature seed is a frequent cause of sterility following interspecific matings in flowering plants. The histological changes leading to failure of fertile ovules of this kind are described in the present paper. Two tissues are primarily involved, endosperm and nucellus. The evidence shows that abortion of the young seed originates in an impaired capacity for growth of the endosperm and that the immediate cause of collapse is starvation of this organ as a result of excessive development of the adjacent maternal tissue.

Since hyperplasia of the nucellus or inner integument is a characteristic feature of the developmental course leading to it, BRINK and COOPER (1939) have designated this type of seed failure somatoplastic sterility. The term was applied, in the first instance, to the collapsing fertile ovules of *Medicago sativa* after self-pollination. It is now shown that essentially the same changes attend seed failure following certain interspecific and intergeneric matings. The occurrence of somatoplastic sterility in association with close inbreeding, on the one hand, and distant outcrossing, on the other, implies that the phenomenon involves processes of basic significance for development of the seed. The essential common element in this apparent diversity of circumstances appears to be the behavior of the endosperm. Elsewhere, the authors (BRINK and COOPER 1940) have discussed reasons for thinking that the endosperm, originating simultaneously with the embryo in an independent fertilization, is of primary importance in the early development of the angiosperm seed. The additional evidence now brought forward is significant in further clarifying the role of this tissue.

The problem acquires a peculiar genetic interest in that the endosperm, even though its duration may be brief and it undergoes little differentiation, is responsive physiologically, nevertheless, to its genotypic composition. The line of descent, of course, is through the embryo. Continuity at the juvenile seed stage, however, appears to depend largely upon the endosperm. The reaction of the latter tissue to its genotype, therefore, is a determining factor in the fate of the zygote.

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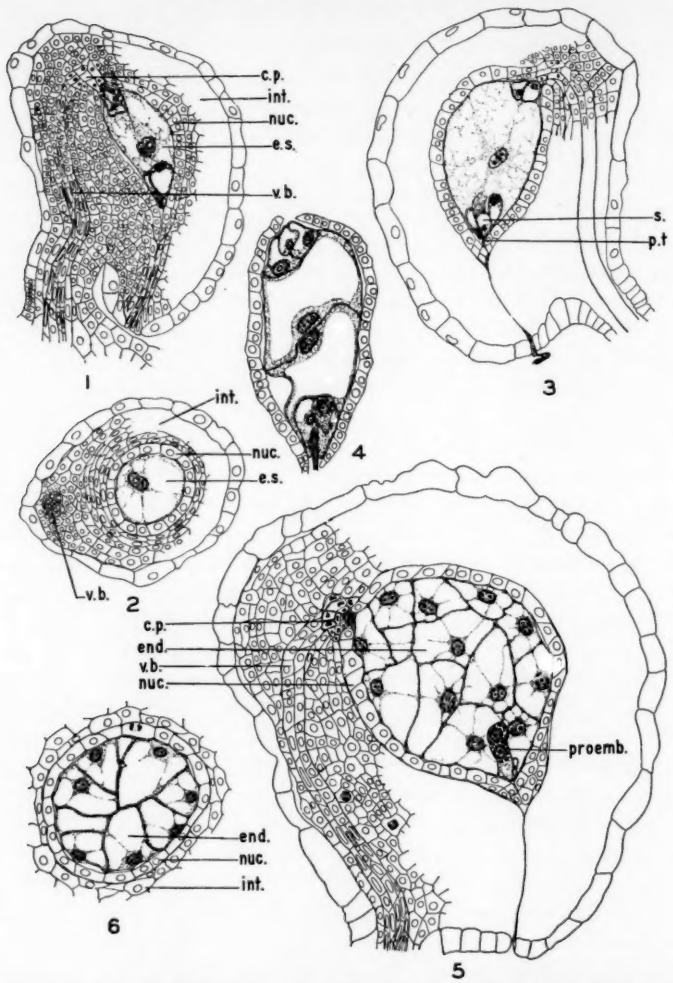


FIGURES 1 to 6.—*Nicotiana rustica* selfed. Figure 1. Longitudinal section through mature ovule; *e.s.*, embryo sac; *nuc.*, nucellus; *c.p.*, chalazal pocket; *int.*, integument; *v.b.*, vascular bundle in funiculus. ×180. Figure 2. Transverse section through mid-region of mature ovule. ×180. Figure 3. Longitudinal section through ovule at 24 hours[1] showing double fertilization. The remains of the pollen tube (*p.t.*) lies in the micropyle and between the synergids (*s*). ×180. Figure 4. Zygote and two-celled endosperm. 24 hours. ×180. Figure 5. Ovule with a two-celled proembryo (*proemb.*) showing the relationship between endosperm (*end.*), chalazal pocket (*c.p.*) and vascular bundle (*v.b.*). The nucellus (*nuc.*) is a single layer of cells. 28 hours. ×180. Figure 6. Transverse section through the mid-region of an ovule containing a two-celled proembryo. A single layer of nucellar cells (*nuc.*) lies between the endosperm (*end.*) and the integument (*int.*). 28 hours. ×180.

[1] Time interval after pollination.

A cytological and histological examination was made in the present study of material collected at stated intervals from self-pollinated *Nicotiana rustica* ($n=24$), a species which is regularly fertile after this type of mating. The course of development was compared with that at like intervals in the crosses, *N. rustica*×*N. glutinosa* ($n=12$), *N. rustica*×*Petunia violacea* ($n=7$) and *N. rustica*×*Lycopersicon esculentum* ($n=12$), in which combinations fertilization may take place but functional seed fails to form.

NORMAL SEED DEVELOPMENT IN *Nicotiana rustica* SELFED

The ovules of *Nicotiana rustica* L. arise from an enlarged placenta which is borne on the inner surface of each carpel. They appear as erect rounded protuberances which later become somewhat pointed. As a result of more rapid growth on the side toward the apex of the carpel, they become bent toward the base and ultimately assume an anatropous position. The body of each ovule comes to lie against the funiculus and the micropyle is directed toward the placenta (fig. 1).

The single integument of the mature ovule is composed of eight to ten layers of cells and completely surrounds the nucellus. The eight-nucleate, seven-celled macrogametophyte occupies the central region of the nucellus and is surrounded by a single layer of nucellar cells. A trace of the vascular tissue from the bundles of the placenta extends well into the funiculus. The relationship of the various structures may be seen in the longitudinal section of the ovule shown in figure 1 and in the transverse section through the mid-region of the ovule as shown in figure 2.

A few cells (15 to 20) at the chalazal end of the nucellus, in the course of the development of the embryo sac, become differentiated into the "chalazal pocket." Such cells in the mature ovule are practically devoid of cytoplasm. Their nuclei are much shrunken and misshapen, and stain heavily. The cells between the pocket and the apical portion of the vascular bundle have not become completely differentiated at this stage.

The egg apparatus at the micropylar end of the macrogametophyte consists of three somewhat pear-shaped cells—the two synergids and the egg. A large vacuole occupies the broad basal portion of each synergid and the apical portion is filled with dense cytoplasm. The small nucleus occupies a position in the cytoplasm immediately above the basal vacuole. A distinct filiform apparatus whose striations arise in the vicinity of the vacuole and extend to the apex is developed in each synergid. These striations consist of dense areas in the cytoplasm which alternate with elongated vacuoles similar to those described for *Lycopersicon esculentum* (COOPER 1931) and *Solanum tuberosum* (REES-LEONARD 1935).

The broad base of the mature egg extends into the gametophyte well beyond the synergids. A large vacuole occupies the pointed apical region of

the egg and the large nucleus remains in the denser cytoplasm in the basal portion of the cell. Numerous starch grains are found in the cytoplasm immediately adjacent to the egg nucleus.

The three large antipodal cells at the chalazal end of the macrogametophyte persist for some time after the initiation of the endosperm. GUIGNARD (1902) likewise observed enlarged and persistent antipodal cells in *Nicotiana tabacum.*

The primary endosperm cell of the macrogametophyte remains binucleate. It is a highly vacuolate cell with a dense strip of cytoplasm extending corelike from the egg apparatus to the antipodals. The two polar nuclei lie closely appressed to each other in the dense cytoplasm near the central portion of the cell. They do not fuse prior to fertilization as is the case in many members of the Solanaceae (*Datura*—GUIGNARD 1902; GLIŠIČ 1928; *Petunia*—FERGUSON 1927; *Lycopersicon*—COOPER 1931; *Solanum*—KRUGER 1932; REES-LEONARD 1935) but remain separate as in *Nicotiana tabacum* (GUIGNARD 1902). No evidence of starch has been observed in the mature macrogamatophyte aside from the grains stored in the egg.

Fertilization takes place approximately 24 hours after pollination (figure 3). Pollen tubes are present in abundance on the placenta of ovaries collected at that interval. The ovules in the apical portion of the ovary show stages of fertilization whereas the basal ovules are unfertilized. SATINA and BLAKESLEE (1935) reported that fertilization takes place in *Datura stramonium* between 22 and 24 hours after pollination. COCHRAN (1938) found fertilization occurring at 42 hours after pollination in *Capsicum frutescens* at a temperature between 70° and 80°F. CLARKE (1940) observed stages of fertilization at 36 hours after pollination in *Solanum tuberosum.*

The pollen tube passes through the micropyle and enters the macrogametophyte between the egg and the synergids. The synergids are not destroyed by the pollen tube as described by GUIGNARD (1902), but remain intact and persist for a time after fertilization, then gradually shrink in size and ultimately disappear. In the course of fertilization one male gamete nucleus becomes closely appressed to the egg nucleus and the other unites with the two polar nuclei.

The first division of the endosperm nucleus takes place shortly after fertilization and cell plate formation occurs, giving a two-celled endosperm. Such a stage of development is shown in figure 4. The male nucleus lies closely appressed to the egg nucleus at this stage. The synergids have not begun to disintegrate as yet and the remains of the pollen tube lie between them. The antipodals likewise are in a turgid condition. The endosperm in the Solanaceae, typically, does not have a free-nucleate stage but is cellular from the beginning (GUIGNARD 1902). Further divisions follow in rapid

succession and the endosperm consists of 16 to 24 large and highly vacuolate cells at the time of the division of the zygote which occurs approximately 4 hours after fertilization. In the ovules containing a two-celled proembryo, the number of cells in the endosperm ranges from 24 to 64 (fig. 5). The nuclei of the single layer of nucellar cells remain centrally located (fig. 6).

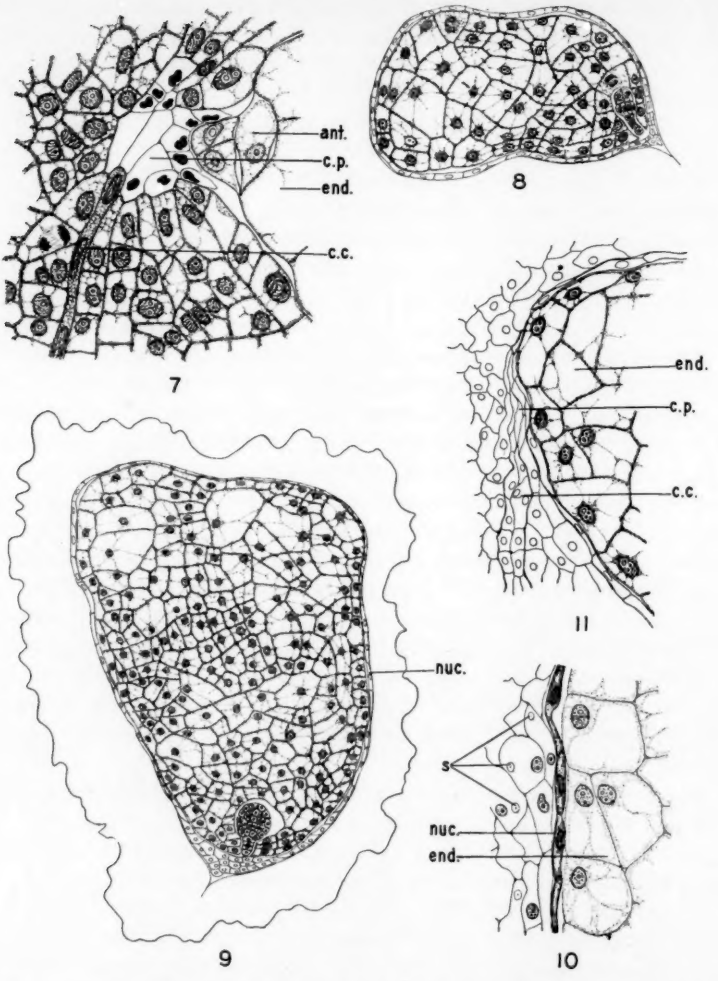
The cells between the apical portion of the vascular bundle and the chalazal pocket undergo differentiation during the course of this development They elongate and become more densely cytoplasmic than the immediately adjacent integumentary cells (fig. 7). Ultimately these cells become differentiated into conducting cells which form a direct connection between the vascular element in the funiculus and the chalazal pocket.

The ovule expands rapidly during the course of the next few days. The cells of the endosperm are actively meristematic and, on the whole, remain large and highly vacuolate, with the exception of those in the immediate vicinity of the embryo which are, usually, somewhat smaller than the average. The nucellar tissue remains one layer of cells in thickness. The individual cells become flattened between endosperm and integument. Their nuclei are centrally located and the cytoplasm stains heavily There is a rapid proliferation of the cells in the integument but the division spindles are oriented so that the structure does not become appreciably thicker as a result of an increased number of cell layers. Longitudinal sections through ovules at 96 and 144 hours after pollination are shown in figures 8 and 9, respectively. The embryos at 96 hours range from 8 to 16 cells plus suspensor. The one shown in figure 8 is composed of 12 cells plus suspensor. The cells of the embryo continue to multiply so that a spherical mass is present at the 144 hour interval.

There is no evidence of starch in either the embryo, endosperm or nucellus at 144 hours after pollination. An occasional starch grain is to be found, however, in the cells of the integument (fig. 10). The relationship between endosperm, nucellus, and integument is shown in figure 10.

The antipodal cells disintegrate during the early course of development allowing the chalazal pocket to come in direct contact with the chalazal end of the endosperm. The cells of the pocket become somewhat elongate and their nuclei disintegrate. As a result, a pad of apparently empty cells forms a direct connection between the endosperm and the conducting tissue of the ovule (fig. 11).

The embryo continues to enlarge but remains spherical until about 15 days after pollination when the first evidences of cotyledonary outgrowths are to be observed. By this time the young seed has grown materially. The relationship between the various parts remains as already described. The cells of the nucellus are further flattened but there is no evidence of their



FIGURES 7 to 11.—*Nicotiana rustica* selfed. Figure 7. Region of the chalazal pocket (*c.p.*) showing the antipodal cells (*ant.*), a portion of the endosperm (*end.*), and the conducting cells (*c.c.*) which are differentiated after fertilization. 28 hours. ×430. Figure 8. Embryo, endosperm and nucellus at 96 hours. ×84. Figure 9. Longitudinal section through an ovule at 144 hours. The nucellus (*nuc.*) remains a single layer of cells. ×84. Figure 10. Portion of the endosperm, nucellus and integument showing an occasional starch grain (*s*) in the cells of the integument. No starch is present in either endosperm (*end.*) or nucellus (*nuc.*). 144 hours. ×430. Figure 11. Chalazal pocket at 144 hours. A direct connection between the endosperm (*end.*) and the conducting cells (*c.c.*) is maintained through the cells of the pocket (*c.p.*). ×180.

disintegration. The few starch grains in the integumentary cells have enlarged considerably and a very few smaller ones are to be seen. There is, as yet, no evidence of starch in other portions of the developing seed.

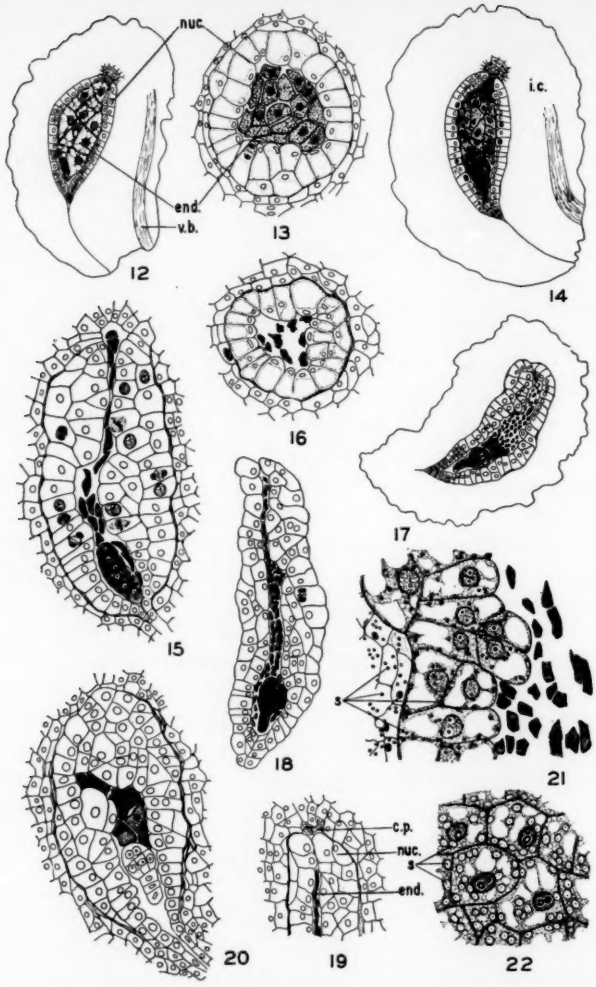
SEED FAILURE IN THE CROSS *N. rustica*×*N. glutinosa*

Many investigators have noted the production of abortive seeds following certain interspecific crosses in the genus Nicotiana. These seeds, upon being planted, either do not germinate or if they do, the young plants die in the seedling stage. No germinable seeds have been obtained following the cross *N. rustica* ($n=24$)×*N. glutinosa* ($n=12$) GÄRTNER (1849) noted the production of weak embryos but the shrunken seed failed to sprout. EAST and HAYES (1912) and EAST (1928) were not successful in obtaining the cross. CHRISTOFF (1928) obtained capsules without viable seed. KOSTOFF (1930) made a cytological examination at various intervals following pollination and found that fertilization was accomplished but that the young seed collapsed after a few days growth.

This cross was made in the present study in order to follow the post-fertilization development and the changes occurring in the embryo, endosperm and adjacent maternal tissue prior to abortion. The pollen tubes reach the ovarian cavity and may be seen growing along the apical portion of the placenta at 48 hours after pollination. Occasional apical ovules are fertilized at this interval. Fertilization, for the most part, however, is accomplished during the succeeding 10 or 12 hours.

Four large and highly vacuolate cells are formed as a result of the first two divisions of the endosperm. Accompanying these divisions the cells of the surrounding nucellus increase greatly in size. A single large vacuole is formed in each cell and the nucleus becomes embedded in the dense cytoplasm on that side of the cell adjacent to the endosperm. The cells of the nucellus enlarge and usually become actively meristematic at about the time the endosperm reaches an 8-celled stage. The exact stage of development at which such meristematic activity is initiated varies somewhat from ovule to ovule. The first evidences of such activity may be manifest in some ovules with a 4-celled endosperm whereas in others it may be delayed until the 16-celled stage is reached. The plane of division in the nucellar cells is, for the most part, parallel to the longitudinal axis of the ovule. The meristematic activity is initiated in those cells nearest the chalazal region of the ovule and on the funicular side of the ovule opposite the vascular bundle.

The nuclear and cell divisions in the endosperm occur at a less rapid rate than is characteristic of the normally developing seed. The cells become smaller as they increase in number and the cytoplasm is less vacuolate after each succeeding division. The number of cells in the endosperm ranges from 8 to 16 at the time of the division of the zygote.



FIGURES 12 to 22.—*Nicotiana rustica*×*N. glutinosa*. Figure 12. Ovule with a two-celled proembryo at 72 hours. The nuclei of the nucellar cells (*nuc.*) have in most instances moved to that side of the cell adjacent to the endosperm (*end.*). A few of the nucellar cells have divided so as to form two layers. ×84. Figure 13. Transverse section through the mid-region of the endosperm (*end.*) and nucellus (*nuc.*) at 72 hours showing an early stage in the hyperplasia of the nucellus. ×180. Figure 14. Ovule at 96 hours. The integumentary cells (*i.c.*) between the chalazal pocket and the apex of the vascular bundle remain undifferentiated. The nucellar cells are in a meristematic condition. ×84. Figure 15. Embryo, endosperm and nucellus at 96 hours. The endosperm is in an advanced stage of disintegration. The cells of the nucellus are in various stages of division. ×180. Figure 16. Transverse section through the mid-region of the endosperm and nucellus at 96 hours.

The majority of the proembryos are in a two-celled stage at the 72-hour interval following pollination (fig. 12). Zygotes and three- or four-celled proembryos are present in occasional ovules. The number of cells in endosperms accompanying two-celled proembryos ranges from 16 to 48. The majority of the 25 endosperms examined accompanying two-celled proembryos possessed from 24 to 48 cells. The individual endosperm cells are smaller than those in *N. rustica* selfed at a like stage of development and have much denser cytoplasm.

Accompanying the slow growth of the endosperm and the hyperplasia of the nucellus and, no doubt, closely associated with it, is the failure of the integumentary cells between the primary vascular element and the chalazal pocket to become differentiated into conducting elements.

The fertilized ovules continue to develop slowly and all ultimately collapse. The abortion may occur at any stage of development ranging from that with a two- to four-celled endosperm plus zygote to that with a multicellular endosperm and a well rounded embryo. Hyperplasia of the nucellus precedes the collapse of the endosperm and this is followed by abortion of the ovule (fig. 13).

If growth of the young seed continues beyond the early stages, the cells of the endosperm become increasingly smaller and their cytoplasm denser (fig. 14). The embryos at 96 hours range in size from four to eight cells plus suspensor. The hyperplasia of the nucellus continues (fig. 15 to 17). In those ovules where there is evidence of endosperm collapse, the embryos have developed more slowly than in adjacent developing ovules and the nucellar cells are conspicuously meristematic (fig. 15).

Many fertile ovules are in stages of abortion at 144 hours after pollination (fig. 17 and 18). The overgrowth of the nucellus has, in most instances, severed the close association between the endosperm and the chalazal pocket (fig. 17 to 20). A densely staining area separates the nucellus from the integument. This is partially accounted for by the heavy end walls of the nucellar cells. There is also an accumulation of stainable materials in the space between the two tissues (fig. 15, 20 and 21).

The first evidences of disorganization of the endosperm are to be seen in the chalazal region. The small densely staining cells become separated

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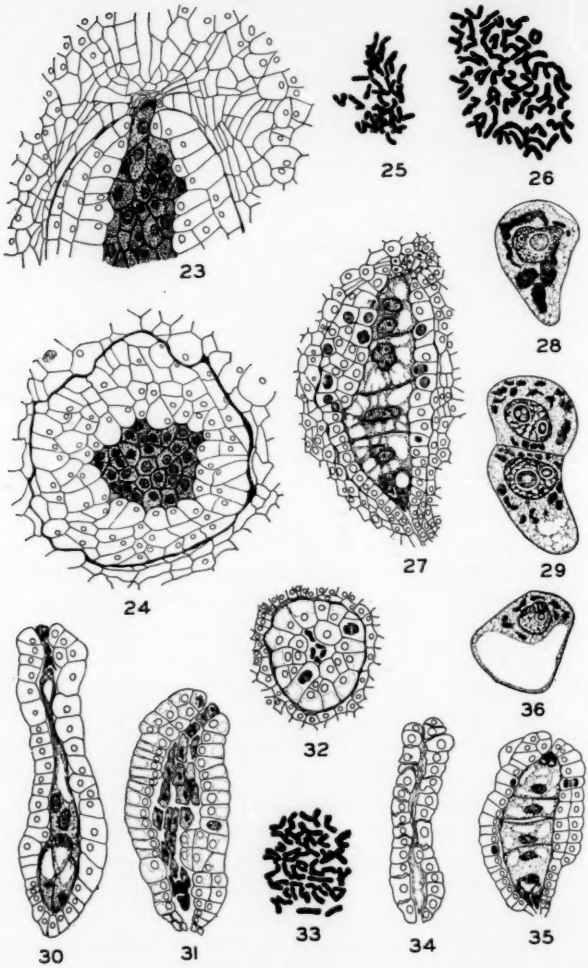
The endosperm cells are in a stage of collapse. ×180. Figure 17. Ovule at 144 hours showing disintegration of the endosperm and hyperplasia of the nucellus. ×84. Figure 18. Embryo, endosperm and nucellus at 144 hours. Embryo and endosperm are disintegrating. There is an extensive overgrowth of the nucellus. ×180. Figure 19. Chalazal region at 144 hours. The extensive overgrowth of the nucellus (*nuc.*) has severed the association between the endosperm (*end.*) and the chalazal pocket (*c.p.*). ×180. Figure 20. Ovule at 144 hours showing extensive hyperplasia of the nucellus. ×180. Figure 21. Portion of endosperm, nucellus and integument at 144 hours. The endosperm is disintegrating. Starch (*s*) is present in the cells of both nucellus and integument. ×430. Figure 22. Nucellar cells at 144 hours showing storage of starch (*s*). ×430.



FIGURES 23 to 26.—*Nicotiana rustica*×*N. glutinosa*. Figure 23. Chalazal portion of endosperm and nucellus as found in an occasional ovule at nine days. The association between the endosperm and the chalazal pocket is much restricted although not entirely interrupted. The adjacent integumentary cells are devoid of contents. There is extensive hyperplasia of the nucellus. ×180. Figure 24. Transverse section through endosperm and nucellus at a stage similar to that shown in figure 23. Note the overgrowth of the nucellus. ×180. Figure 25. Somatic metaphase in an embryo cell with 36 chromosomes. ×1550. Figure 26. Equatorial plate in an endosperm cell with 60 chromosomes. ×1550.

FIGURES 27 to 34.—*N. rustica*×*Petunia violacea*. Figure 27. Zygote, endosperm and nucellus at 72 hours, showing meristematic condition of the nucellar cells. ×180. Figure 28. Zygote with closely appressed gamete nuclei. 72 hours. ×840. Figure 29. 2-celled proembryo at 72 hours.

from one another (fig. 15 to 17), and their nuclei become disorganized. This disorganization advances toward the embryo until ultimately the entire endosperm collapses. The cytoplasm of the cells of the embryo (fig. 15) becomes increasingly dense as the disorganization of the endosperm advances (fig. 18) and there is an accumulation of degenerative products. The general appearance of both endosperm and embryo is one of starvation.

Starch is stored in the cells of the integument and nucellus surrounding a collapsing endosperm (fig. 21). The integumentary cells have many starch grains and those of the nucellus are packed with starch (fig. 22) before the complete collapse of the ovule whereas only an occasional starch grain is present in the cells of the integument and there are none in the nucellar cells of the normally developing seed (fig. 10).

An occasional ovule is found to be still developing at 9 days after pollination. There is an excessive overgrowth of the nucellus in such ovules (fig. 23 and 24). The association between the endosperm and the chalazal pocket, in these instances however, has not been completely destroyed. A narrow, finger-like projection of the endosperm maintains the association for a time and the endosperm and embryo continue growth (fig. 23). Such development is carried on at the expense of the integumentary cells adjacent to the chalazal pocket. These cells become totally devoid of nuclear and cytoplasmic materials. A transverse section through the mid-region of the nucellus and endosperm of such an ovule reveals the extensive hyperplasia of the nucellus (fig. 24). These ovules collapse at later stages of development and probably appear as shrunken seeds in the mature capsule. All the ovules in material collected 15 days after pollination were in stages of abortion.

The numbers of chromosomes in the mitotic figures of dividing embryo and endosperm nuclei are 36 and 60, respectively (fig. 25 and 26). These numbers afford definite proof that the development of the hybrid embryo and endosperm is initiated by double fertilization.

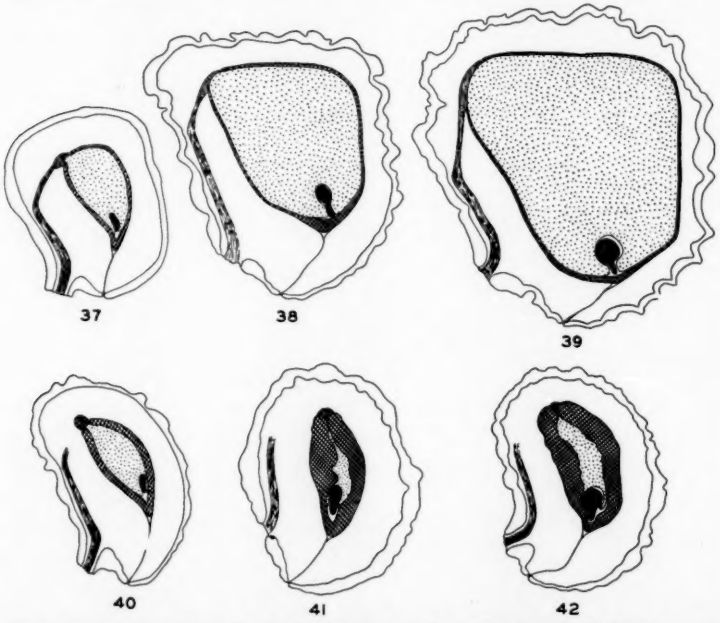
COMPARATIVE DEVELOPMENT OF NORMAL AND COLLAPSING SEEDS

Comparison of post-fertilization stages following the two types of mat-

×840. Figure 30. Unfertilized embryo sac and nucellus. 72 hours. ×180. Figure 31. Proembryo, endosperm and nucellus. 96 hours. Proembryo and endosperm are in an early stage of disintegration. The cells of the nucellus have enlarged and show stages in mitosis. ×180. Figure 32. Transverse section through endosperm and nucellus showing hyperplasia of the latter. 96 hours. The endosperm is in an advanced stage of disintegration. ×180. Figure 33. Polar view of a mitotic figure in the endosperm. 55 chromosomes are present. ×1550. Figure 34. Unfertilized embryo sac and nucellus. 144 hours. ×180.

FIGURES 35 and 36.—*N. rustica*×*Lycopersicon esculentum*. Figure 35. Zygote and four-celled endosperm. The cells of the surrounding nucellus are in a meristematic condition. 96 hours. ×180. Figure 36. Zygote with closely appressed gamete nuclei. ×840.

ings, that is, *Nicotiana rustica* selfed and *N. rustica*×*N. glutinosa* emphasizes the wide divergence in type of development. Fertilization occurs between 20 and 24 hours following selfing. Stages of ovule development at 28, 96 and 144 hours after self-pollination are diagrammatically depicted in figures 37, 38 and 39. The embryo and particularly the endosperm develop rapidly. The nucellar cells become flattened and form a thin and gradually shrinking layer between endosperm and integument. A direct connection between the endosperm and the vascular element entering the ovule is quickly established through the persisting cells of the chalazal pocket by differentiation of the integumentary cells between it and the distal portion of the vascular bundle.



FIGURES 37 to 39.—Stages in the development of the fertilized ovule of *Nicotiana rustica* selfed at 28, 96 and 120 hours respectively after pollination. The relationship between the endosperm, chalazal pocket and vascular bundle of the ovule is shown. (semi-diagrammatic).

FIGURES 40 to 42.—Stages in the development of the fertilized ovule of *N. rustica* after pollination with *N. glutinosa* at 72, 96 and 144 hours respectively. The integumentary cells between the apex of the vascular bundle and the chalazal pocket do not become differentiated into conducting cells. Note the overgrowth of the nucellus and at later stages the disassociation of the endosperm and chalazal pocket due to this overgrowth. (semi-diagrammatic).

In contrast, pollen tube growth is slower following crossing and fertilization does not occur until between 48 and 60 hours after pollination. Cor-

responding stages following hybridization, in terms of embryo growth, are diagrammatically shown in figures 40, 41 and 42. The course of development is conspicuously different from that just reviewed in normal *rustica* ovules. The endosperm is much less developed and the distal portion of the vascular strand fails to differentiate. Instead of regressing, as in the normal seed, the nucellus forms a considerable amount of tissue which may completely surround the endosperm, thus interrupting the latter's contact with the chalazal pocket. The cells of the pocket then shrink and collapse. The connection of the endosperm with the vascular element of the ovule is thus much restricted and may be completely blocked.

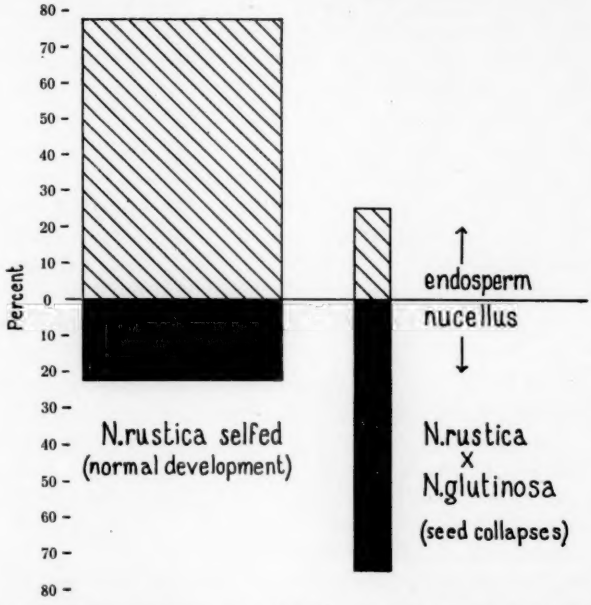


FIGURE 43.—Relative development of endosperm and ncuellus associated with approximately 20-celled embryos. The areas of the rectangles are proportional to size of endosperm plus nucellus. The relative development of these two tissues is represented by the portions above and below the zero line.

Measurements of relative development of endosperm and nucellus associated with approximately 20-celled embryos plus suspensor show the great disparity between the two types of ovules. The average total volume of endosperm and nucellus in *N. rustica* selfed, as determined from serial sections of six ovules, is about five times that following the species cross (fig. 43), where measurements from ten ovules were recorded. Likewise, there is a marked difference in the proportion of endosperm and nucellus

following the two types of matings. In normal *rustica* ovules 77 percent of the volume is endosperm and 23 percent is nucellus whereas in the hybrid ovules, on the average, only about 25 percent is endosperm and 75 percent is nucellus. The percentage of endosperm in the hybrid ovules ranged from 15 percent to 35 percent in the ten ovules examined.

These measurements indicate that, in the seeds which continue development, the endosperm establishes a dominant role. Collapse of the hybrid seeds is associated with a limited growth of this tissue, incomplete development of the vascular strand and a pronounced hyperplasia of the nucellus.

SEED FAILURE IN *N. rustica*×*Petunia violacea* CROSSES

The cross *Nicotiana rustica*×*Petunia violacea* has been described as one wherein the pollen tubes reach the ovary, penetrate the micropyle and induce parthenocarpy (KOSTOFF 1930). Our observations, however, are not in agreement. The Petunia pollen tubes reach the Nicotiana ovary at about 44 hours after pollination and are found growing along the placenta at the 50 hour interval. Double fertilization occurs shortly thereafter and the fertilized ovules, on the average, contain a zygote and an eight-celled endosperm at 72 hours after pollination. Figure 27 is a longitudinal section through an ovule showing the zygote and a four-celled endosperm. The endosperm nuclei have divided and cell plates are being formed leading to the eight-celled stage. The cells of the nucellus are in a meristematic condition and in one region the nucellus has become three to four layers of cells in thickness. Figure 28 is a detailed drawing of the zygote. The male gamete nucleus is closely appressed to the egg nucleus. Occasionally, two-celled proembryos are to be seen at this time (fig. 29). Such embryos are accompanied by endosperms which range from 16 to 24 cells in size. In adjacent ovules wherein fertilization has not occurred, the embryo sacs are in an early stage of disintegration. The unfertilized ovules show no evidence of nucellar stimulation (fig. 30).

Both embryo and endosperm develop slowly thereafter whereas active meristematic activity continues in the nucellus. At 96 hours after pollination endosperm and embryo are usually in a stage of collapse (fig. 31 and 32). Two- and three-celled proembryos are commonly present and an occasional four-celled proembryo is to be observed. Starch is being stored in the cells of the integument and nucellus. All pollen tubes have completely disintegrated by this time.

The stages of ovule development were followed up to 9 days after pollination. Collapse of the endosperm and embryo is usually complete by the sixth day. The central portion of the ovule, with the exception of a narrow core of collapsing endosperm, becomes completely filled with nucellar cells due to the continued proliferation of this tissue. All of the cells of the ovule

with the exception of those of the disintegrating endosperm and embryo become gorged with starch.

The fusion of the gametic nuclei in the zygote (fig. 28) and the presence of 55 chromosomes on endosperm equatorial plates (fig. 33) is conclusive evidence that double fertilization takes place. BIRAGHI (1929) in the cross *N. rustica* var. *Brasilia*×*Petunia* sp. observed dividing endosperm cells at 72 hours after pollination but no zygote. The endosperm collapsed at later stages (102 and 168 hours after pollination). The embryos accompanying such abortive endosperms were believed to have originated as a proliferation of the nucellus. BIRAGHI did not mention having observed a hyperplastic condition of the nucellus in ovules containing a collapsing endosperm.

KOSTOFF (1930), on the other hand, found no evidence of fertilization. Proliferation of nucellar cells following the cross was described and considered to be due to stimulation by the pollen tube. No material was examined prior to seven days after pollination. He described the presence of a pollen tube in the micropyle at that interval. As already noted, pollen tubes had completely disintegrated in the material collected at 96 hours after pollination and no pollen tubes were present in any of the material of this cross taken at later intervals. Likewise, the embryo sacs and nucelli of unfertilized ovules at 96 hours (fig. 34) are in such an advanced stage of disintegration that it is doubtful if any stimulation would be possible at that time or later.

KOSTOFF and PROKOFIEVA (1935) pollinated *N. rustica* with a mixture of equal parts of *N. rustica* and *Petunia violacea* pollen and obtained plump and abortive seeds in equal proportions. The abortive seeds had very small embryos and a multilayer nucellus. Such seeds were assumed to have developed as a result of cross-fertilization.

THE COLLAPSE OF SEEDS FOLLOWING *Nicotiana rustica*×*Lycopersicon esculentum* CROSSES

The cross *Nicotiana rustica*×*Lycopersicon esculentum* was attempted in order to determine (1) if fertilization would be accomplished following such a wide cross and (2) if seed failure involved the same series of changes as discussed above. Nicotiana styles were decapitated just below the stigma and a mass of crushed Lycopersicon stigma was placed on the cut surface. This mass was then covered with an abundance of Lycopersicon pollen. The pollen germinated readily and the pollen tubes grew slowly along the stylar canal. Large numbers of pollen tubes are present on the apical portion of the placenta in material collected 72 hours after pollination but there is no evidence of fertilization. In the material collected 96 hours after pollination a number of ovules had been fertilized. The most common type

of fertilized ovule at this time is one wherein a zygote and a four-celled endosperm are present (fig. 35). The stages range from early post-fertilization to a zygote with an eight-celled endosperm.

The zygotes, especially those at early post-fertilization stages, reveal evidences of a fusion of the two gamete nuclei (fig. 36). The cells of the surrounding nucellus are highly vacuolate and their nuclei have moved to that side of the cell adjacent to the endosperm mother cell. These cells become somewhat enlarged and are in a meristematic condition in all the ovules containing two-celled endosperms. The nucellus consists of two and in some instances three layers of cells in those ovules with four- and eight-celled endosperms. These larger endosperms are in an early stage of collapse. There is no evidence of meristematic activity in any portion of the unfertilized ovules. Although the evidence is less complete, it appears very probable that the failure of fertile ovules to develop into seeds following this cross is essentially like that in the preceding cases.

DISCUSSION

Sterility due to post-fertilization failure of the ovule is a rather common phenomenon in plants, including numerous cultivated forms. Geneticists often encounter it in distant crosses, and it has long been known to occur in varying degrees within species. Heretofore, however, this form of sterility has not been precisely defined and little was known concerning its cause. This is due partly to incomplete, and sometimes,conflicting observations. The authors believe, however, that the principal difficulty in analyzing and explaining it has been the lack of a satisfactory working theory regarding development of the seed. The focal point of morphogenetic interest has been the embryo, relatively little attention being accorded the other parts of the seed. Double fertilization remained an enigma and, so long as this was true, there could be no clear perception of the significance of the endosperm. The preoccupation of investigators with the embryo, while understandable on morphological grounds, failed to yield a satisfactory basis for interpreting development of the seed in functional terms. The reason for this is that it left in the background the endosperm, which from a dynamic point of view, now appears to be fundamentally important. The authors (Brink and Cooper 1939, 1940) have recently shown that the interrelations of the various tissues of the growing seed become intelligible only if a primary role in early ontogeny is attributed to this anomalous second product of the fertilization process.

It is the behavior of the endosperm which affords the principal clue to an understanding of seed failure in the interspecific matings now under discussion. Retarded development of this tissue sets in motion a series of changes within the young seed leading, eventually, to collapse. The changes

conform to the general pattern characteristic of somatoplastic sterility, as earlier described in *Medicago sativa* (BRINK and COOPER 1939).

Matings between distantly related forms, which are followed by fertilization but in which no germinable seeds develop, are advantageous for a study of this kind because the fate of the ovule is determinate. That is to say, it can be predicted beforehand that all ovules becoming fertilized by foreign sperm will fail. In contrast, the control matings—in this case, *Nicotiana rustica* selfed—lead to a nearly full complement of germinable seeds. The essential anatomical features of somatoplastic sterility are especially clear in the three different Nicotiana crosses studied. The phenomenon, therefore, can now be characterized more fully than was possible from the Medicago data alone.

Previous to the collapse of the seed, the hybrid proembryos and embryos differentiate normally. There may be some reduction in size as compared with the control, *N. rustica*, but it is not marked. It is a noteworthy fact that of the four principal tissues in the young seed—embryo, endosperm, nucellus and integument—the embryo is least altered in form and mass by hybridization. If the changes leading to failure of the seed stem from the embryo at all, this structure shows no evidence of it. The ultimate death of the embryo seems clearly to be a secondary phenomenon occasioned by the milieu. LAIBACH'S (1929) demonstration that the half-grown embryo in a collapsing seed of *Linum austriacum* after fertilization with *L. perenne* may be dissected out, cultivated for about two weeks in a nutrient medium, and eventually reared to a mature plant is further evidence that lethality in cases of this kind is not necessarily inherent in the zygote itself.

The hybrid endosperm, on the other hand, grows differently from the very early stages onward. Unlike the embryo, it quickly responds visibly to the radical genotypic change occasioned by hybridization with a widely different species. The rate of cell division is slower and the cells are smaller and less vacuolate than in *N. rustica* selfed. No mitotic irregularities are observable. The difference lies rather in reduction in number and size of cells and in failure of the protoplasts to differentiate in the usual way. The pronounced departures from the norm in these respects are illustrated in figures 12 and 14.

Even with these differences in growth of the endosperm, however, it is conceivable that a functional seed might eventually develop were not significant collateral changes in the adjacent tissues also in progress. Failure is not due, therefore, to an autogenous "breakdown" of the endosperm any more than it is a result of abnormal behavior of the embryo. The effect is indirect; and it is at this point that the present material has proved especially useful in establishing the sequence of events ultimately leading to collapse.

Parallel with the retarded growth of the young hybrid endosperm, two well-marked departures from the normal course of development appear in the adjacent tissues. (a) The nucellus, initially one cell-layer in thickness and remaining so in *N. rustica* selfed, becomes meristematic. Beginning on the funicular side of the ovule in the region of the apex of the vascular bundle, the cells become abnormally large and undergo a rapid proliferation. This continues until a multilayer jacket of nucellar tissue is formed. (b) The integumentary cells between the end of the vascular strand of the ovule and the chalazal pocket, adjacent to the endosperm, fail to differentiate into conducting elements. This is in marked contrast with the normal Nicotiana seed in which a direct vascular connection to the chalazal pocket at the boundary of the endosperm is early established.

The subsequent behavior of the hybrid seeds is briefly recounted. The hyperplastic nucellus often completely occludes the endosperm by overgrowing the chalazal end. Thus walled off, the endosperm is isolated from its source of food supply. Although the endosperm shows signs of increasing starvation, there is no visible disintegration of the tissue up to this stage. Breakdown, however, soon begins. The cells at the chalazal end collapse first and the disorganization progresses toward the embryo. By the time the latter is involved, growth of the seed has ceased. Rarely does development of the *N. rustica*×*N. glutinosa* hybrid seed continue beyond six days after pollination.

Figure 23 represents an exceptional case which serves to clarify the phenomenon further. This *N. rustica*×*N. glutinosa* seed was still growing at nine days. Its relatively long persistence appears to be due to a finger-like portion of the endosperm projecting beyond the enveloping nucellus into the chalazal pocket. The depleted appearance of the adjacent integumentary cells indicates that the continued growth is at the expense of this tissue. The vascular connection to the chalazal pocket is no more complete than in the seeds failing several days earlier. The significant fact brought to light is that the hyperplastic nucellus is an effective block to the movement of nutrients into the endosperm. The maintenance of even a narrow gap through the nucellus to the chalazal pocket significantly prolongs the life of the seed. It is in such an anatomical relationship that one might expect to find a basis for collapse of the seed after matings leading to failure at various later stages between fertilization and maturity.

The abundant formation of starch in the integumentary and nucellar cells of the seeds destined to collapse indicates that failure is not due to lack of a nutrient supply. Foods move into the hybrid seeds and, so far as the carbohydrates are concerned at least, in amounts sufficient for considerable storage. Abortion occurs not because nutrients are absent but because they are abnormally distributed within the seed. The direct cause of failure in

the hybrid seeds appears to be starvation of the endosperm through a more or less complete obstruction of the immediate line of supply by the overgrown nucellus. One may think of the hyperplastic nucellus both as a physical barrier to the inward movement of materials and as converting to its own use foods which would otherwise pass to the endosperm and embryo.

An important point which can now be established beyond any reasonable doubt is the time relationship between endosperm collapse and the hyperplasia of the adjacent maternal tissue. Impairment of the endosperm follows so quickly upon overgrowth of the adjacent inner integument in the aborting fertile ovules of *Medicago sativa* that the order of the two events is discoverable in a relatively few cases only (BRINK and COOPER 1939). Disintegration of the endosperm in the Nicotiana hybrid seeds, on the other hand, may not begin until several days after hyperplasia of the nucellus is clearly apparent. It may be concluded with certainty, therefore, that overgrowth of the local maternal tissue is a forerunner and not a resultant of the endosperm breakdown.

A discussion at this stage of the physiology of somatoplastic sterility must be rather general in character. Certain functional relationships, however, may be inferred from the histological evidence. We have set forth some of these in seeking an interpretation of the significance of double fertilization in the development of the seed (BRINK and COOPER 1940). These earlier conclusions can now be amplified and extended.

Somatoplastic sterility is essentially a malnutritional phenomenon associated with the genotypic diversity of the tissues within the seed. The angiosperm ovule at the mature embryo sac stage, unlike that in many gymnosperms, is characteristically lacking in food reserves. The continuing absence of storage products shows that the nutrient level remains low for a significant period after fertilization. Now, the latter event stimulates to more or less rapid development not only the two immediate products of syngamy but also the much more extensive surrounding maternal tissues. Competition for a limited food supply thus arises, the outcome of which seems to depend largely upon the physiological balance existing between the endosperm and the adjacent maternal cells. There is no evidence that in early ontogeny the embryo plays an active role in this regard. Since parent sporophyte and endosperm are of independent genetic origin the issue may turn more or less directly upon their respective hereditary constitutions. The fertilization stimulus is apparently non-specific, growth beginning in the surrounding tissues even after distant outcrossing. Development of the endosperm, however, is a function of the latter's genotype. It has been shown, for example, that the rate of nuclear division in the alfalfa endosperm is depressed by inbreeding (BRINK and COOPER, 1940); and the present evidence corroborates a fact already apparent from other studies (*cf.*

BOYES and THOMPSON 1937) that after interspecific hybridization growth of the endosperm may depart sharply from the norm. Within the present frame of reference, development of a fertilized ovule into a mature seed, therefore, depends upon whether the genotype of the endosperm possesses certain functional properties relative to those of the associated maternal genotype. The general requirements which the endosperm genotype must meet may be inferred from the facts at hand.

The collapse of fertilized ovules in *Medicago sativa*, which is about five times as frequent following self-fertilization as after cross-fertilization, is found to be associated with a reduced rate of endosperm growth (BRINK and COOPER 1940). At nine intervals, from the two-celled proembryo to four-celled true embryo plus suspensor stage, the embryos in the inbred series had fewer endosperm nuclei associated with them than did the embryos of the crossbred series. The Nicotiana interspecific hybrid seeds show a similar retardation in endosperm development. This low rate of endosperm growth appears to be the primary factor in somatoplastic sterility. It is occasioned in the inbred alfalfa ovules, presumably, by the bringing together in homozygous form of certain unfavorable recessive genes and in the hybrid Nicotiana seeds by the inharmonious reactions of widely different genoms. A basic condition for maintenance of growth in a fertile ovule is that the endosperm be so constituted genetically that it develops with sufficient aggressiveness to maintain an ascendancy over the adjacent maternal tissues.

The balance between endosperm and nucellus, and hence, the amount of somatoplastic sterility, on this hypothesis might be shifted by altering the physiological condition of the mother plant. In our experiments, however, we have attempted only to vary the properties of the endosperm by introducing into it male nuclei of diverse types.

The mechanism by which rate of growth of the endosperm affects the surrounding tissues of the young seed is largely a matter of speculation. The simplest hypothesis seems to be that the partition of nutrients moving into the seed depends directly on the relative rates of growth of the tissues inside and outside the embryo sac. The synthetic processes are probably similar in all the growing cells, so that the same nutrients will be in concurrent demand. As we have pointed out elsewhere, under these conditions of parallel growth, the limited supply of nutrients will be shared between the endosperm and the adjacent maternal structures in proportion to the velocity of growth in these respective tissues. Continued development of the seed requires that a balance be maintained which will insure nourishment of the parts inside and outside the embryo sac. If the balance is upset by failure of the endosperm to keep pace with growth in the extensive surrounding tissue, the latter gains the ascendancy, the endosperm and its

nursling, the embryo, are overgrown and starved, and the seed eventually collapses (BRINK and COOPER 1939).

This interpretation, while accounting for the endosperm-nucellus relationship, fails to explain the non-differentiation of the terminal portion of the vascular strand in hybrid seeds. At the fertilization stage the vascular element within the ovule extends about half way to the chalazal pocket. Normally, the gap is closed in *Nicotiana rustica* about 48 hours later by differentiation of the intervening integumentary cells. The establishment of a more or less complete and direct vascular connection to the endosperm must be considered a critical step in the development of the seed. A final answer to this important aspect of the problem, of course, cannot be given. One might reasonably suppose, however, that a rapidly growing endosperm receiving its food materials through the limited portion of its surface that is in contact with the chalazal pocket would create a rather well defined line of flow. Differentiation of vascular tissue along this path would seem to be connected, in some manner, with the directed movement of nutrients. A slower growing hybrid endosperm, on the other hand, would cause a lower initial diffusion potential relative to the vascular supply, which might soon be nullified as the nucellus became meristematic. Since the nucellus extends around the endosperm, sac-like, its transformation into a very actively growing tissue would tend to spread the incoming nutrients widely. The main channel between vascular bundle and endosperm, once disorganized in this way, would not be expected to reestablish itself and complete development even though the seed persists for several days. This is what is observed.

Several earlier studies of seed failure have been made in which the observations indicate somatoplastic sterility as the cause of collapse. A comprehensive review of this work cannot be given here, but brief mention will be made of a few of the more informative cases. Unfortunately, several otherwise significant investigations have not dealt adequately with the critical early post-fertilization events.

RENNER (1915) examined the early stages of seed development following the cross *Oenothera muricata* Venedig × *Oe. biennis* and found very few nuclei in the endosperm as compared with *Oe. muricata* selfed. The embryo, following cross-pollination, develops for a short time and then disintegrates. The nucellar cells at the chalazal end of the endosperm, in many instances, expand noticeably, such expansion never being found in normally developing *Oe. muricata* seeds. When *Oe. Lamarckiana* is selfed, three types of seeds are produced. In those ovules with normally developing embryo and endosperm the nucellus becomes a thin and shrinking layer between endosperm and integument, whereas in those ovules with abortive embryos and endosperms and in others without any evidence of either of these structures the nucellus is not shrunken but in many instances is considerably enlarged.

KRAUS (1915) observes that the most important and inclusive kind of sterility in the orchard fruits is that due to post-fertilization disturbances. Wide variation occurs in the extent to which seeds develop. Failures occur at all stages in ontogeny and, of course, many seeds mature normally. KRAUS thinks that the failure of seed development is due to some upset in the nutritive processes and suggests that abnormal development of the somatic tissue may be a factor. The problem in the orchard fruits is undoubtedly complex. Further study of early post-fertilization behavior of the ovule, however, might help to clarify it. Somatoplastic sterility appears to be an important factor. BRADBURY (1929), for example, in a study of sterility in the sour cherry, presents rather clear evidence for its occurrence (her Plate LIV, figs. 24 and 25). Her illustrations show that at the "third drop" stage a normally developing seed possesses a well differentiated embryo, a large, cellular endosperm and a thin layer of nucellar tissue. In collapsing seeds, on the other hand, the embryo is smaller, the endosperm is weakly developed and the nucellus has become definitely hyperplastic.

The endosperm and embryo following the cross *Datura stramonium*×*D. metel* disintegrate between six and fifteen days after pollination. SATINA and BLAKESLEE (1935) observed that these structures in the hybrid seeds develop slowly and that the epithelial cells which surround the embryo sac become enormous in size.

BOYES and THOMPSON (1937) have shown that in crosses between Triticum species and in Triticum×Secale matings, in which the seed is either abortive or badly shrunken, the endosperm is markedly abnormal in development. The embryos, on the other hand, are much less affected. It would be interesting to know the condition of the nucellus and the behavior of the vascular tissue in these cereal hybrids.

Extensive histological studies of seed development following species crossing in Nicotiana have been reported by KOSTOFF (1930). Hyperplasia of the nucellus is noted as a regular feature of the seeds which abort and is attributed by the author to a stimulus arising from foreign substances introduced by the pollen tube. KOSTOFF considers that failure of the seed is due to an immunity reaction between the genotypically diverse endosperm and the adjacent maternal tissues. This explanation appears to be unnecessarily complex and fails to account for certain essential facts. In the first place, the deposition of materials at the boundary between endosperm and nucellus, which KOSTOFF cites as evidence of antigen-antibody activity, is not a constant feature of this kind of seed failure, as BOYES and THOMPSON (1937) point out. Nor does the hypothesis explain why hyperplasia of the nucellus is initiated on the side of the endosperm adjacent to the vascular element. KOSTOFF's interpretation breaks down entirely when considered in relation to the evidence on seed failure after self-fertilization

in Medicago (*cf.* BRINK and COOPER 1939). Here the elements united in fertilization originate within the plant toward which, on KOSTOFF's hypothesis, they should show an immunity reaction. Yet the course of developmental events leading to collapse of the inbred alfalfa seed is essentially like that occurring after outcrossing to distantly related species.

SUMMARY

1. A comparative histological study of seed development in *Nicotiana rustica* following two classes of matings (a) self-pollination and (b) outcrossing to *N. glutinosa*, *Petunia violacea* and *Lycopersicon esculentum* is reported. Selfing leads to normal seed formation. In the distant crosses, on the other hand, the seeds fail at early stages of growth.

2. Double fertilization is found to have occurred in all the seeds wherein development begins, and both embryo and endosperm start growth. Fertilization is delayed 24 hours or more in the outcrosses, however, and is sharply reduced in frequency.

3. The hybrid embryos grow and differentiate normally, or nearly so, up to the time of the collapse of the young seeds. The accompanying endosperms, on the other hand, develop differently from the very early cell divisions onward.

4. The endosperm develops rapidly after self-fertilization of *N. rustica*, forming numerous, relatively large, highly vacuolate cells. The hybrid endosperm cells divide slowly and become increasingly smaller and denser as development proceeds.

5. Whereas the nucellus in the normally developing *N. rustica* seed remains one cell layer in thickness, this tissue becomes actively meristematic and multilayered in the hybrid seeds.

6. The hyperplastic nucellus may completely occlude the endosperm by overgrowing the chalazal end. The maintenance of even a narrow gap through the nucellus to the chalazal pocket significantly prolongs the life of the hybrid seeds.

7. The vascular bundle in the ovule of *N. rustica*, at the mature embryo sac stage, extends about half way from the funiculus to the chalazal pocket. The gap is closed in a few hours after self-fertilization by differentiation of the intervening integumentary cells into vascular elements. Such differentiation fails to occur in hybrid seeds even though the latter lived eight to ten days.

8. Previous to abortion, the nucellus and integument of the hybrid seeds become packed with starch, indicating that failure does not result from lack of supply of nutritive materials.

9. The final collapse of the seed begins with breakdown of the endosperm which is initiated in the chalazal region and progresses toward the embryo.

More or less marked hyperplasia of the nucellus precedes the first signs of disintegration of the endosperm.

10. Failure of the hybrid seeds is considered to be due, primarily, to retarded development of the endosperm. The effect on fertility, however, is indirect, secondary changes in other parts of the seed being involved.

11. The immediate causes of collapse are occlusion, or partial occlusion, of the endosperm resulting from hyperplasia of the nucellus, and failure of the vascular bundle to complete its growth and differentiation. These histological abnormalities provide a structural basis for starvation of the endosperm and the enclosed embryo. Starvation results from maldistribution rather than lack of nutrients within the ovule.

12. The course of development leading to collapse of the seed following interspecific-hybridization is essentially like that often found in *Medicago sativa* after close inbreeding. Occurrence of the phenomenon under these diverse circumstances indicates that somatoplastic sterility is of wide significance.

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GENETIC BEHAVIOR OF EUCHROMATIC SEGMENTS INSERTED INTO HETEROCHROMATIN

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ONE type of viable chromosomal rearrangement frequently induced by X-ray treatment is a translocation in which a piece of a chromosome is taken from its normal position and inserted into some other part of the chromosomal complex. When such a rearrangement is intrachromosomal the name "transposition" is applied and when it involves two chromosomes it is called an insertional translocation. A number of transpositions and insertional translocations have been described. In this paper results will be given of studies made of five such cases in which euchromatic segments of the X chromosome of *Drosophila melanogaster* are inserted into heterochromatic regions.

Below is a list of X chromosome genes mentioned in this paper with their location on the linkage map given in parentheses (BRIDGES 1938b): *bi*, bifid (6.9); *cm*, carmine (18.9); *ct*, cut (20.0); *cv*, crossveinless (13.7); *cx*, curlex (13.6); *dm*, diminutive (4.6); *dx*, deltex (17.0); *ec*, echinus (5.5); *fa*, facet (3.0); *g*, garnet (44.4); *N*, Notch, deficiency for *fa* (3.0); *peb*, pebbled (7.3±) *pn* prune (0.8); *rb*, ruby (7.5); *rg*, rugose (11.0), *rst*, roughest (1.7), *rux*, roughex (15.0), *sc*, scute (0.0+), *scp*, scooped (18.5±), *shf*, shifted (17.9), *sn*, singed (21.0), *vs*, vesiculated (16.3), *w*, white (1.5), *y*, yellow (0.0).

DESCRIPTIONS AND EXPERIMENTAL DATA

All five rearrangements which will be described here were found in routine experiments carried on with the purpose of inducing changes in the Notch and cut loci of the X chromosome by X-ray irradiation applied to males. All changes involving the Notch locus are given the experiment number 264 and those affecting cut the experiment number 268. Individual changes in each group are numbered in the order in which they were found; thus 264-58 indicates the 58th change affecting the Notch locus. Salivary chromosomes map designations refer to BRIDGES' 1938a map for the X chromosome and BRIDGES' 1935 maps for autosomes.

Notch 264-58.—*N* 264-58 was found on April 11, 1938 among the offspring from a mating between a white cut female and a treated yellow male. The original female had notched wings and white eyes with a few red spots. Cytological tests made by SUTTON (1940) show that a section of 20 bands from 3B3.4 to 3D5.6 inclusive is inserted in an inverted order in the chromocenter of 3L proximal to 80C. By subsequent breeding two

lines were isolated, one having the deficiency in the X chromosome and the other with a duplication in 3L. Cytological analysis indicates that nothing was lost in the transfer, that is, that all bands which are missing in the Notch region are inserted in the 3L heterochromatin. Genetic tests show that the deficiency involves the *w*, *rst*, *fa* and *dm* loci, while the inserted segment, when together with recessive alleles, shows spotting for *w*, *rst* and *fa* and the recessive mutant change in the *dm* locus. Neither the deficiency nor the duplication affects *pn* which is to the left of *w* nor *ec* which is to the right of *dm*, indicating that these two loci are outside the limits of the *N* 264-58 change.

The duplication is viable both in the males and in the females. Crosses were made between females heterozygous for different lethals in the Notch and white loci and males carrying the duplication to determine if it covered the lethal effect. In all cases where the lethal effect is covered hyperploid males should appear carrying the lethal in the X chromosome and the duplication in 3L. The following lethals were studied:

*N*264-32, a deficiency for four bands, 3C4 to 3C7 inclusive;

*w*258-45, a deficiency for a single band, 3C1;

*N*264-8, *N*264-40, *N*264-46, *N*264-47, all lethal Notches but without any cytologically detectable deficiencies;

*N*264-34, *N*264-53, *N*264-69, all translocations with one break adjacent to Notch and the other in the euchromatin of an autosome, none of them showing any detectable deficiency in the Notch region;

*N*264-62, a translocation with one break between 3C7 and 3C8 and the other in the heterochromatin of 2R, heterozygous with *w* and *fa*, shows mottling for both loci;

*N*264-48, an inversion with one break following 1B6 and the other between 3C7 and 3C8;

*N*264-52, an inversion with one break between 3C3 and 5 and the other between 20B1.2 and 20C1.2, showing spotting for *rst*, *fa*, *dm*, *ec* and *bi;* and

*N*264-63, a transposition of a piece from 13C to chromocenter into Notch region between 3C7 and 3C9.

The lethal effect was covered only in the case of *N*264-53. In that case *yN*264-53/In dl-49, *y Hw* m^2 g^4 females were crossed with *w* Dp264-58 males and the following F_1 offspring were obtained:

Females:		108
Males:	In dl-49, *y Hw* m^2 g^4	43
	y	13
	w	11
	w-mottled	12

In dl-49 are the normally expected males, *w*-mottled and *w* are non-dis-

junction males with or without the duplication respectively and *y* males have the Notch X chromosome covered by the duplication. While the wild-type/duplication males are fully fertile the *N*/Dp males obtained in this experiment are sterile. Moreover under normal conditions one would expect the *N*/Dp class to be one half of the dl-49, *y Hw* m^2 g^4 class while actually it is significantly smaller. This indicates that even in this instance the duplication did not entirely cover the lethal effect of *N*264-53.

Notch 264-85.—*N*264–85 was found on April 26, 1939 among the offspring of a cross between *y sc w* females and X-rayed wild-type Swedish-b males. The original female had notched wings and white eyes with red spots. Cytological analysis made by SUTTON (1940) shows a complex translocation in which an euchromatic section of X from 3C1 to 6A1.2 inclusive is inserted in its normal order in the chromocenter of the fourth chromosome between 101F and 102A. In addition there is a reciprocal translocation between 2R and X. The distal segment of 2R from 60A5 to the tip is attached to X at 6B1.2 and the distal portion of X from 3B3.4 to the tip is attached to 2R at 60A3.4. The light doublet 6A3.4 is unaccounted for and it may be either deficient or it may be obscured in the preparations because of the distortion caused by the rearrangement. If this doublet were present in the segment inserted in the chromocenter it would be very difficult to find it.

The section of the X chromosome inserted into the heterochromatin of the fourth includes 145 bands. The inserted piece segregates freely from the remaining X chromosome and consequently females deficient (or hypoploid) for the segment are expected to occur as well as both females and males hyperploid for the segment. Hypoploid females were not found indicating that the deficiency for such a long sector in this region of the chromosome is a dominant lethal. Hyperploids were found both among females and among males. Hyperploid females are fertile while hyperploid males are sterile showing that this long duplication upsets the physiological balance in males to such an extent that sterility is produced. A few 264-85 males were obtained from cultures kept at 28°C. These males showed mottling for *w*, *cx*, *cv* and for rough eyes (presumably *rst*, *fa*, *rg* and *rux*).

Crosses were made between Notch females which were heterozygous for the insertion and males carrying recessive alleles in the loci present in the inserted segment. All Notch females from such crosses have one normal X chromosome carrying the mutant and another which is involved in the translocation. These females showed mottling for *w*, *rst*, *fa* and *dm* located in the distal end of the insertion and for *rg*, *cx*, *cv*, *rux* and *vs* in the proximal end. This mottling is due to instability of the loci involved. The loci of *ec*, *bi*, *peb* and *rg* in the central portion of the insertion were found to carry wild-type alleles.

Since the hyperploid males are sterile, experiments could not be made to determine whether or not the duplication covers the lethals located in the duplicated section of the chromosome.

Notch 264-86.—*N*264-86 was found on September 18, 1939 as a single Notch female among the offspring of a cross between a *y pn* female and an X-rayed wild-type Swedish-b male. Cytological analysis made by SUTTON (1940) shows that a section of the X chromosome from 3C7 to 3E5 inclusive is inserted in its normal order into heterochromatin of 4 at 101F. This section segregates freely from the remaining body of the X chromosome and thus a deficiency and a duplication may be isolated. The deficiency is viable in heterozygous females and the duplication is viable both in females and in males.

Cytogenetic study indicates that the inserted piece is one band longer than the deficiency, which can be accounted for by the assumption that the chromosome was split at the time of the breakage and that both strands did not break at the same level (DEMEREC and SUTTON 1940).

The inserted segment includes 17 bands. When heterozygous for *fa* and *dm* it shows mottling for both loci. Notches *N*264-94 and *N*264-97, neither of which shows any cytological deficiency, were tested with the duplication to determine if their lethal effect would be covered. The results of these tests were negative. Also *N*264-53, which was covered by *N*264-58 duplication was tested with the duplication 264-86. From the cross *y N*264-53/dl-49, *y Hw* m^2 g^4×*w spl* Dp264-86 following offspring were obtained:

females: 364	
males: dl-49, *y Hw* m^2 g^4	176
w spl	41
w spl/Dp	30

Since *y* males did not appear it is evident that this duplication does not cover the lethal effect of *N*264-53. The *w spl* and *w spl*/Dp classes are due to non-disjunction.

Notch 264-100.—*N*264-100 was found as a single female on December 13, 1939 among the offspring of a cross between *y sc w* females and X-rayed wild-type Swedish-b males. This female had white eyes with red spots. Cytological analysis made by SUTTON (1940) shows that a piece of the X chromosome from 3C1 to 4B3.4 inclusive is inserted into the chromocenter of 3 between 80C and 81F. This region is unmapped on BRIDGES' chart. On the available cytological figures it has not been possible to determine in which arm of the chromosome the insertion is located.

The inserted piece involves 45 bands. Notch females heterozygous for *w, rst, fa, dm* and *ec* show mottling in all these loci. Genetic tests indicate that hypoploid females are viable and fertile. Hyperploid males have not

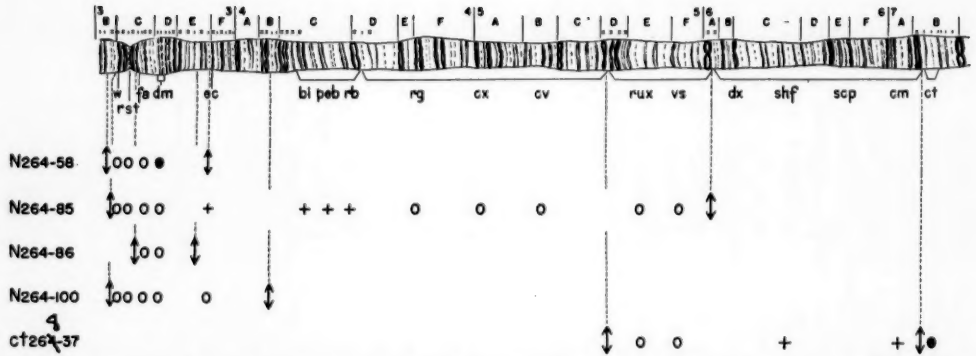

FIGURE 1.—Section of the BRIDGES (1938a) salivary chromosome map on which the position of loci is indicated. Each short line above the map represents a band and two horizontal lines indicate a doublet. The limits of each of the five insertions are shown by arrows, o's stand for loci which show mottling, full •'s for loci in which recessive stable change has occurred and crosses indicate loci with wild-type alleles. (Last entry in the first column should read ct 268–37 instead of ct 264–37.)

been found among a large number of flies examined in the various tests. Since these tests include crosses with a number of different stocks as well as cultures raised at low and at high temperatures the non-appearance of males suggests that males carrying the duplication are not viable.

*Cut 268-37.—ct*268-37 was found on November 7, 1939 as a single *ct* female among the offspring from a cross between *ct*^{n} *g* females and X-rayed *y* males. Breeding tests showed that this change in the cut locus was connected with a lethal effect. This may be due either to a lethal allele of *ct* or, since this is an insertional translocation, the lethal may have been induced in connection with any one of the three breaks. Cytological study made by SUTTON (1940) shows that a piece of the X chromosome from 5D3.4 to 7B1.2 inclusive is inserted in an inverted position in the heterochromatin of 3L between 40F and 41A. In the inserted piece all bands which are missing in the X chromosome are accounted for. The insertion includes a section of 76 bands.

Hyperploid females and males and heterozygous hypoploid females are viable and fertile. Hypoploid females show a strong minute bristle character and have a lower viability. Genetic tests show that *rg*, *cx* and *cv* which are on the left side of the rearrangement and *sn* which is on the right side are not affected and thus are not located in the inserted segment. It has been already mentioned that *ct* shows a recessive change. It is known that the cut locus is represented by bands 7B3-5 (DEMEREC and HOOVER 1936) and since one of the breaks occurred adjacent to and to the left of 7B3 the locus *ct* is not located in the inserted segment. Tests with *rux* and *vs* show that recessive changes producing mottling have occurred in these loci. Tests with *shf* and *cm* indicate that the insertion carries wild-type alleles at these loci.

DISCUSSION

Experimental evidence presented in the foregoing pages is summarized graphically in figure 1. That figure shows the pertinent section of the salivary map of the X chromosome which has been copied from the revised Bridges' map (BRIDGES 1938a). The position of loci is shown as determined by work in this laboratory which is still unpublished.

An extensive study of mottling in the loci of the white-Notch region is now under way, and the results will soon be ready for publication. In the course of this study mottling has been observed in the following loci: *pn*, *w*, *rst*, *fa*, *dm*, *ec*, *bi*, *rg*, *cx*, *cv*, *rux* and *vs*. Results indicate that mottling occurs in conjunction with a chromosomal rearrangement when a certain locus is brought into the proximity of a specific region of a chromosome. As has been shown by SCHULTZ (1936) and later confirmed by a number of investigators heterochromatin is effective in inducing mottling. My studies indicate (Demerec in press) that not all regions of the heterochromatin

are equally potent, and also that certain sections of the heterochromatin do not induce mottling at all. On the other hand these studies suggest that all autonomous loci might show mottling given a suitable environment.

The diagrams in figure 1 demonstrate that all loci included in the insertions *N*264-86 and *N*264-100 show mottling. In the case of *N*264-58 all loci included in the insertion are affected, but only *w*, *rst* and *fa* exhibit mottling while *dm* shows a recessive mutant change. Such recessive changes are frequently observed in chromosomal rearrangements involving euchromatic segments. As a rule the locus which happens to be adjacent to the break is affected, and only in exceptional cases is the locus changed which is separated from the break by several bands. The length of the sensitive region within which a break may induce a change varies with the locus. A majority of the loci studied have a very short sensitive region.

The available evidence indicates that the sensitive region of the *dm* locus is of medium size and therefore the right break of *N*264-58 may well be within it and may be responsible for the recessive change in *dm*. In that case since the insertion is inverted the region of the heterochromatin of 3L between 80C and the centromere is effective in inducing mottling while the region to the left of 80C is not effective. However, in salivary gland chromosomes that region has the characteristic appearance of heterochromatin. A similar situation is found in *ct*268-37 where a section of the X chromosome is inserted in an inverted position into heterochromatin of 3L to the right of 80C in approximately the same position as in *N*264-58. Here again the region close to the centromere induces mottling while the region to the left of 80C does not. Since the cytological evidence indicates that the *ct* locus is not included in the inserted segment, the recessive change in *ct* is probably caused by a break in its proximity and a subsequent fusion to euchromatin.

A good illustration of the extent of the influence of heterochromatin is found in *N*264-85. Here a long segment of 145 bands is inserted in an inverted position in the heterochromatin of the fourth chromosome. Mottling is induced in the loci on each side of the inserted segment but the loci in the center are not affected. On the right side the effect stops between *rb* and *rg*. It is known that *rg* is located in the section between the 22nd and 81st band from the right end and therefore the effect on the right side extends through at least 22 bands and may reach as far as 80 bands It appears probable that the effect on the right side extends through a longer distance than the effect on the left side. It is of interest to note that the right side is attached close to the centromere. On the left side the effect stops between *dm* and *ec*. Since *dm* is located in the 13th and 14th band from the left break and *ec* is the doublet represented by the 27th and 28th band from the break, the effect on the left side extends through at least 14 bands and it stops before the 27th band is reached.

Evidence is available showing that the effect in this region of the chromosome may spread to a longer section than 14 to 26 bands. In *N*264-52, which we have in our collection, a section from 3C4 to 20B1.2 inclusive is inverted and thus the *N* region is brought into the proximity of heterochromatin. In this case mottling is evident in *rst*, *fa*, *dm*, *ec* and *bi* and therefore the effect extends at least 50 bands from the break.

The material presented here indicates that certain regions of heterochromatin are not effective in inducing mottling. The data show that the region to the left of 80C was ineffective in both *N*264-58 and *ct*268-37. In these cases that region is moved away from the centromere by insertions. It may be argued that the increase in the distance from the centromere may be responsible for the non-appearance of mottling. However, the inserted segment in *N*264-85 is much longer than either in *N*264-58 or *ct*268-37 and in spite of that the heterochromatin is effective in producing mottling. I am inclined to think that the quality of the heterochromatic region as well as its relation to the centromere and its quantity are all important factors in determining mottling. The evidence in support of this view was discussed in a paper read at the Seventh International Genetics Congress in Edinburgh (DEMEREC in press).

Changes which occurred in the inserted segments are best visualized by analyzing biological effects produced by them. The most striking and at the same time the most characteristic effect is mottling. As an illustration I will use the mottling observed at the white locus. In all cases mentioned in this paper the females heterozygous for the insertion and the white gene have eyes prevailingly cream or very light cherry with smaller or larger spots colored dark cherry or red (wild type). Judging from the appearance and the distribution of spots it seems probable that red spots originate through changes from light color into dark color which have occurred during the development of the eye. If this is expressed in terms of genes it may be said that in light regions the action of the wild-type allele of the white gene, which was present in the segment before insertion, is partially or totally suppressed. Total suppression produces white background and partial suppression light color. In red spots the activity of the gene is again fully restored. It is important to note that in the case of large red spots all facets in a spot are red, indicating that when the restoration of activity occurs it persists among the daughter cells.

The question now arises concerning the mechanism involved in the suppression and the restoration of the activity of the affected genes. Two possibilities are evident. (1) We may be dealing here with real chemical changes in genes which are unstable and revert to the original state. In this case it would be assumed that the change in the position of the locus produced a reversible chemical change in the gene and that reversions occur during the ontogeny of the fly whenever conditions are suitable. (2) It is also possible

that because of the shift in position the action of the gene is suppressed without any change in the chemical constitution of the gene itself. In such a case also certain physiological conditions arising during the ontogeny of the fly would reestablish the activity of the gene and thus bring about the development of wild-type spots. I intend to discuss the evidence in favor and against these two possibilities in another paper where experiments dealing with 35 cases of mottling will be described. In this paper I am only considering the facts which are specific to the material described here.

From a theoretical standpoint the experiments in which the covering effect of duplications was tested are of particular interest. Tests with duplication *N*264-58 showed that of 12 lethals connected with the Notch phenotype only one, namely *N*264-53, was covered by the duplication and in addition one lethal white tested was not covered. One of the Notches and the white were cytologically detectable deficiencies while all other Notches had a full complement of bands in salivary chromosomes. Similarly neither of the three lethal Notches tested which included also *N*264-53 was covered by *N*264-86 duplication. These results show (1) that these duplicated segments inserted in the heterochromatin were not only unable to cover a physical deficiency for a small part of the duplication but in the majority of cases they were unable to cover a biological deficiency which is expressed as a lethal; (2) that there is a difference between the Notches in regard to lethality and (3) that there is a difference between similar duplications in their ability to cover the lethal effect of loci which are present in duplication.

The inserted segment of *N*264-58 shows mottling for *w* and for *fa*, which is considered an allele of *N*, but was not able to cover a deficiency affecting either of these two loci. It has been shown by POULSON (1940) that the abnormalities in development which bring about the death of the hemizygous flies are evident in very early embryonic development, during blastoderm formation, in the case of Notches and somewhat later in the case of white lethals. This indicates that in early embryonic stages the function of white and facet-Notch genes in the inserted segment of *N*264-58 is not adequate to cover deficiencies in these loci. However, the wild-type spots which appear on the eyes of flies indicate that their function is normal in some of the cells during late stages of development.

Another interesting fact brought out in these experiments is the difference in viability of duplications. It has been shown that males carrying the *N*264-85 duplication are viable although they are sterile, while the males carrying the *N*264-100 duplication have never been found and presumably this combination is lethal. Both duplications have identical left limits. The *N*264-100 segment includes 45 bands and the *N*264-85 segment includes in addition to these same bands 100 others. It is evident that the lethal effect

of *N*264-100 cannot be due to the unbalancing effect of the total number of genes present in the duplicated segment, since the total number of genes in N264-85 duplication is much larger. The lethal effect could be brought about only through the unbalance of the genic system caused by the activity of the genes located in the duplicated segment, which activity is determined by the position of the segment in the gene system.

SUMMARY

Description is given of five insertional translocations in which euchromatic pieces of the X chromosome are inserted into heterochromatin. Figure 1 summarizes the results of cytogenetic analysis.

As a rule the loci brought into the proximity of heterochromatin show mottling. When the inserted segment is long, as in the case of *N*264-85 where it includes 145 bands, the loci on both ends of the segment show mottling while the loci in the center are not affected.

In *N*264-58 and *ct*268-37 segments are inserted in 3L to the right of 80C in an inverted position, and in both cases the loci farthest from the centromere do not show mottling.

A number of Notches and one lethal white were tested with duplications *N*264-58 and *N*264-86. In all but one Notch the duplication did not cover the lethal effect.

The inserted segment in *N*264-85 includes all bands of the segment in *N*264-100 and in addition 100 bands more. In males the duplication for *N*264-100 is lethal while the duplication for *N*264-85 is not, indicating that in this case the length of the segment is not responsible for the lethal effect.

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TERMINAL DEFICIENCIES IN THE X CHROMOSOME OF DROSOPHILA MELANOGASTER

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IN THE course of cytogenetic studies on the X chromosome in *Drosophila melanogaster* conducted in this laboratory by DR. M. DEMEREC, several terminal deficiencies have been found.

Three of these have already been described by DEMEREC and HOOVER (1936) and it is the purpose of this paper to describe others and to put forward evidence that they are really terminal deficiencies in which a broken chromosome has failed to rejoin either in the old or in a new combination, so that a new chromosome end is formed at the breakage point.

The standard nomenclature for genes and chromosomal changes in Drosophila (as in Drosophila Information Service No. 10) will be used throughout as follows: genes in the X chromosome with positions on genetic map—*ac*, achaete (0.0+); *bb*^{l}, bobbed lethal (66.0); *car*, carnation (62.5); *f*, forked (56.7); *Hw*, hairy wing (0.0+) *l J1*, lethal J1 (−0.0); *N*, Notch (3.0); *pr*, prune (0.8); *sc*, scute (0.0+); *spl*, split (2.9±); *w*, white (1.5); *y*, yellow (0.0).
genes in other chromosomes—
2nd chromosome: *bw*, brown; 3rd chromosome: *e*, ebony; 4th chromosome: *ey*, eyeless; *M-4*, Minute-4; *sv*^{n}, shaven-naked.
chromosomal changes—
Df, deficiency; Dp, duplication; T, translocation

METHODS

Slides of the salivary gland chromosomes of female larvae heterozygous for the different changes were prepared by the acetocarmine technique.

The equipment used in analysing the slides consisted of a 90 × 1.3 N.A. apochromatic objective, an oil-immersed 1·4 N.A. condenser, 12.5 × compensating oculars, and a Bausch and Lomb research lamp with the green Wratton filter number 61.

Cytological analyses were based throughout on BRIDGES' (1935 and 1938) maps of the salivary chromosomes.

ANALYSIS OF THE CHANGES

1 Df(1) 260.10.

This deficiency came from a mating of *y sc w* females with wild Swedish-b males which had been treated with X-rays. It was identified in an F_1 female which was phenotypically yellow, indicating that the *y* locus of the paternal

X chromosome was changed or deficient. The *ac* locus was found to be affected also.

Females carrying the mutant X chromosome (*y ac*) over normal *y sc w* were found to be heterozygous for a deficiency involving bands 1A1 and 2 of the salivary chromosomes. The appearance of the figures suggests that the whole tip to the left of 1A3 has been lost.

Males with the deficient X are viable and fertile, and must therefore carry the normal allele of the *lJ1* locus, which, according to MULLER, lies to the left of the *y* locus. Similarly, Df(1) 260.5 (DEMEREC and HOOVER 1936) has four bands missing, but is perfectly viable and fertile.

Data obtained in this laboratory show that the genes *y* and *ac* must both be located in the region 1A 5–8 and that their change was in this case due to "position effect." The work of MULLER (1935), and cytological results of MULLER, PROKOFYEVA and RAFFEL (1935) do not exclude the possibility that *lJ1* also is located in this region. This would allow deficiencies such as these to lack the extreme tip of the X chromosome and yet to carry the normal allele of the *lJ1* locus.

2. Df(1) 260.19.

This deficiency was picked up from cytological observations on a stock of STERN ($\widehat{yy}/g^2$ *B*) and must have occurred spontaneously. It resembles 260.10, cytologically, the break being in the same position between 1A2 and 3. It differs genetically as the loci of *y* and *ac* are unaffected. The males are viable and fertile.

3. Df(1) 260.25.

Genetical data. The stock carrying this deficiency was derived from an F_1 female carrying a normal *y sc w* chromosome and an X chromosome from an irradiated Swedish-b male. This female was phenotypically scute, and when mated with *y sc w* males she gave the following offspring:

	Females	Males
sc	11	—
y sc w	15	13
y sc	13	—
sc w	6	8

This progeny shows three peculiar features: (1) There is apparently an abnormally high crossover value for the *y-sc* and/or *sc-w* regions, giving relatively large numbers of *y sc* and *sc w* flies. (2) The *y sc* type is inviable in the hemizygous condition (no *y sc* males). (3) The mutant *sc* type is not recovered in the hemizygous condition (no *sc* males).

These results could be accounted for on the supposition that the tip of X including the y^+ gene had been translocated to some other chromosome region where it segregated with comparative freedom from *sc* and *w*, while

the break at the tip of X had caused a mutation in the *sc* locus accompanied by a recessive lethal change. The *sc* and *y sc w* classes would then represent the parental types (*sc* failing to appear among the males on account of the lethal). The *y sc* flies would be heterozygous for a deficiency including the *y* locus, this deficiency probably being lethal in males, which would in any case fail to survive on account of the lethal *sc* mutation. The *sc w* class would carry two normal *y sc w* chromosomes together with a duplication for the tip region including the y^+ gene.

On closer examination the *sc* and *sc w* flies showed a variegated body and bristle color, predominantly wild-type with patches of yellow, suggesting that the y^+ gene was inserted in some chromocentral region. Mosaicism of wild-type and mutant tissue is characteristic of such changes (SCHULTZ 1936).

A test mating with *y ac* showed that variegation of the achaete character also occurred. The break in the X was to the right of *y* and *ac*, therefore, but probably to the left of *sc*, because it showed no variegation.

In order to locate the translocated segment carrying y^+ and ac^+, a cross was made between females of a stock, (*yvf; bw; e; ey*) and *sc w* males assumed to carry a normal *y sc w* chromosome and a duplication for y^+ ac^+. The F_1 consisted of 309 *y v f* females and two wild-type females with 369 *sc w* males and one *y v f* male. The F_1 females receive both their X chromosomes (attached) from their mother, while the males receive their X chromosome from the father; the other chromosomes are distributed at random. Since all females (with two exceptions) were yellow and all males (with one exception) were wild-type with respect to the yellow locus, the duplication clearly segregates with the X chromosome. The exceptions could be accounted for by the rare formation of detached X's by crossing over in the female parent, whereby one son received a *y v f* chromosome from the mother, and two daughters received only one *y v f* chromosome from their mother, the second X chromosome with the y^+ duplication being derived from the father. These data showed that the tip region of the X was transposed to the chromocenter of the same chromosome.

A linkage test was made with genes at the proximal end of the X chromosome to determine the position of the duplication in respect to these genes. The back cross *y* Dp/*y f car* $bb^1 \times y$ *f car* bb^1 gave the results shown in table 1. In this experiment the flies were raised at 25°C in order to get crossover values comparable with the standard values.

From this mating all classes of females homozygous for bb^1 are lethal and thus lacking. Crossing over between *f*, *car*, and Dp is therefore shown adequately in the males only. The crossover value between carnation and the duplication calculated from the males is 3.41 ± 0.40 percent, so that it is not detectably different from the crossover value for *car*-bb^1 (3.5 percent).

Crossing over between the duplication and *bb*^{1} could be detected only in the females. Since double crossovers between *car* and *bb*^{1} would not be expected, the single yellow among these shows that the duplication is genetically to the right of, and very close to, *bb*^{1}, giving less than 0.1 percent of crossing over.

A cross was made between females carrying the deficient X chromosome and a normal X marked by the dominant gene *Hw* and males of the *lJ1 sc J1* stock in which the lethal change is covered by a deleted X chromosome. From this cross (Df/*Hw*×*lJ1 sc J1*−del 24), half of the F_1 females should have the deficient X chromosome and a *lJ1 sc J1* chromosome,

TABLE 1

Offspring of backcross y Dp/y f car bb^{1}×*y f car bb*^{1}.

FEMALES			MALES		
non-crossovers	Dp	1184	non-crossovers	Dp	1002
	y f car bb^{1}	—		*y f car*	815
region 1	*f* Dp	36	region 1	*f* Dp	69
(*f-car*)	*y car bb*^{1}	—	(*f-car*)	*y car*	43
region 2	*f car* Dp	35	region 2	*f car* Dp	33
(*car-bb*^{1})	*y bb*^{1}	—	(*car*-Dp)	*y*	32
region 3	*y*	1	regions 1, 2	*y f*	—
(*bb*^{1}-Dp)	*f car bb*^{1} Dp	—		*car* Dp	3
Total		1256	Total		1997

and only half of these should carry the covering deletion. If the deficiency includes the normal allele of *lJ1*, the *sc* flies which lack the deletion will be inviable, and the ratio of *Hw* to *sc* in the F_1 females will exceed 1:1. If the normal allele of *lJ1* is still present in the deficient chromosome, all *sc* females should be viable whether they carry the deletion or not, and the ratio of *Hw* to *sc* should be 1:1.

A very great excess of *Hw* females was actually found, the total numbers being 171 *Hw*:17 *sc*. It is evident that there is some factor disturbing the ratios other than the failure of the *sc* females which lack the deletion, and the test is therefore rather unsatisfactory. It is not apparent, however, that the normal allele of the *lJ1* locus is still present in the deficient chromosome.

On the genetical evidence, the deficiency may include all genes to the left of *sc*, and may therefore be a deficiency for the entire tip of the X chromosome.

Cytological data. Female larvae heterozygous for the *sc* change were obtained from the mating *sc*/*y sc w*×*y sc w*. Their salivary gland chromosomes showed an abnormality at the tip of X, where all bands to the left

of 1B 3.4 were missing in one of the strands. A further peculiarity was the presence of a nucleolus-like structure at the deficient tip, which looked as if material had oozed out from the end of the chromosome (Plate 1, fig. C). This phenomenon is common as a casual occurrence in figures where a partial or complete break has occurred (not induced by irradiation) in one of the "weak spots" of the chromosomes (for instance, in region 11A); but in this case it appeared consistently in every figure. This is regarded as evidence that the newly-constituted chromosome tip consists of the naked broken end, without any other material attached to it.

It proved impossible to locate the translocated segment 1A1 – 1B1.2 at the X chromocenter, so it is not known whether it is inserted in an intercalary position or attached terminally to the short arm.

It was considered desirable to test the capacity of the new end for rejoining with other broken ends, and for this purpose viable males carrying the tip deficiency were obtained by mating the *y sc* females (heterozygous for the deficiency) with Dp(1) 118, thus introducing a fragment, made up of the extreme distal and proximal regions of the X, which covered the lethal effect of the scute change and of the deficiency.

Males carrying the 260.25 deficiency and Dp 118 were treated with X-rays at a dosage of 4000r, and mated to wild Canton-S females. Slides were made from F_1 female larvae, which had both a normal X and the irradiated deficient X.

In 102 pairs of glands, 27 aberrations were obtained with a total of 63 breaks, but in no case had the broken tip of the deficient X become attached to one of the newly broken ends. It is apparent, therefore, that healing has taken place, so that the tip does not tend to rejoin as is usual within a short time after breakage has occurred.

4. T(1; 4) 258.53.

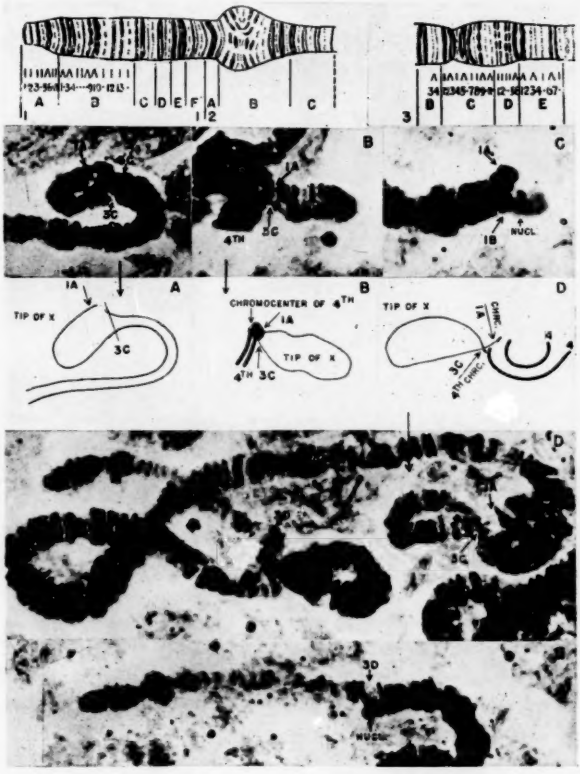
The existence of eye color variegation in an F_1 female from the mating of *y sc w* by X-rayed Swedish-b male indicated a translocation of the white locus (band 3C1 in the salivaries) to the chromocenter.

Observation of the salivary chromosomes showed that the tip of the X

LEGEND FOR PLATE 1

Top: Diagrams of regions of X chromosome from BRIDGES' salivary chromosome map.

Below: A. T(1; 4) 258.53, haploid tip of normal X bent back so that distal region (1A) pairs with distal end (3C) of deficient X (see diagram). B. T(1; 4) 258.53, tip of X attached to fourth chromocenter, distal end (1A) also pairing with chromocenter (see diagram). C. Df(1) 260.25 lower strand, deficient for 8 distal bands, shows nucleolus-like structure extending beyond normal tip of upper strand. D. T(1; 4) 264.113 at left, haploid tip of normal X and deficient strand ending at 3D; at right, tip of X attached to 4th chromocenter, distal end also bent back to pair with chromocenter (see diagram). E. T(1; 4) 264.113, haploid tip of normal X and, above, deficient strand ending in a small nucleolus-like structure.


A
B
C
D
E
F
1
2
3
3C
4TH
1A
1B
NUCL
TIP OF X
CHROMOCENTER OF 4TH
4TH 3C
CHROM.
4TH CHROM.
3D

up to and including 3C1 was attached to the chromocenter, apparently at the base of the fourth chromosome, but there seemed to be two normal fourth chromosomes, and there was no foreign material attached to X distal to 3C2.3 (Plate 1, figs. A, B).

The salivary configurations suggested two possibilities: (1) that the tip of X was attached to the short arm of the fourth, in which case it was possible that a piece of this arm, so small as to be practically invisible, had been translocated to the distal end of the X; or (2) that the tip of the X had been attached to the long arm of the fourth, near the proximal end, in which case the broken distal end of 4R must have been lost, the broken tip of the X remained unattached at 3C2.3, and non-disjunction of the fourth chromosome must have occurred subsequently to restore the second normal fourth chromosome.

A cross with the fourth chromosome recessive *sv*^{a} decided between these alternatives. The F_1 from the mating Df+w^{mot}−4/*y Hw w*; +^{sv}/+^{sv}×*w*; *sv* gave the following classes:

w^{mot}+	27
w^{mot} *sv*	13
w+	32

The presence of *sv* flies in the F_1 in the class which also carried the X-4 chromosome responsible for white mottling, can be explained only by the second alternative. The break in the fourth chromosome must have been proximal to the *sv* locus, so that the X-4 chromosome lacks the wild-type allele of this locus. The three fourth chromosome centromeres apparently segregate more or less at random, and some of the gametes carry no normal fourth, but only the X-4 chromosome, deficient for the *sv* locus. These gametes are responsible for the w^{mot} *sv* flies appearing in F_1 from the mating with the *sv* stock. These flies must be haploid for all of the fourth chromosome except the proximal region.

The subsequent discovery of Minute flies in the T(1;4) 258.53 stock supported this explanation. These Minutes must be due to the same independent segregation of the X-4 chromosome from the two normal fourths, and they are comparable with BRIDGES' M-4, which is haploid for a part of the fourth chromosome owing to a deficiency (BRIDGES 1935).

In view of this evidence, the end of the abnormal X, broken to the left of 3C2.3, can be considered as having reconstituted itself as a normal end without the attachment of any previously existing end structure.

5. T(1;4) 264.113.

This aberration was obtained in a Notch F_1 female from a mating of *y pn* females with irradiated Swedish-b males. It closely resembled the previous one, 258.53 (Plate 1, fig. D) but differed cytologically in two respects:

(1) the break in X occurred between 3C9·10 and 3D1; (2) the unattached broken end, terminating to the left of 3D1, frequently showed a nucleolar structure, similar to that found in Df(1) 260.25, except in that it was much smaller. This exudation sometimes obscured the distal bands of 3D (Plate 1, fig. E).

The cytological difference in the position of the break corresponded with a genetical difference. This stock showed a Notch mutation and mottling of *w* and *spl*, all of these loci being transferred to the fourth chromocenter, whereas in 258.53 only the *w* locus was affected.

DISCUSSION

The special behavior of chromosome ends, namely the fact that they do not as a rule become attached permanently to one another or to newly broken ends, but persist as ends, has led to a supposition that they must have a special structure which conditions their behavior. The name of telomere has been applied to this terminal structure, and it has been supposed that reproduction of the telomere maintains the identity of the chromosome end.

The literature provides occasional examples of behavior which is inconsistent with this view of the chromosome ends. Thus, although STADLER (1932) remarked on the absence of terminal inversions in maize, such an inversion has subsequently been found in *Drosophila ananassae* (KAUFMANN 1936). A further example of anomalous behavior of chromosome ends has been found by MCCLINTOCK (1939), who described chromosomes derived from the breaking of dicentric anaphase bridges during meiosis in maize. These chromosomes, though not deficient, have broken ends. In the endosperm the broken ends of half chromatids undergo a repeated cycle of fusion, dicentric bridge formation, and subsequent rupture. In the sporophytic tissue, however, no anaphase bridges are found. It is apparent that one of the chromosome set in this tissue was derived from breakage of a dicentric bridge, because of the absence of a characteristic knob; and it is therefore evident that the broken end has healed so as to form a normal, permanent chromosome end.

MULLER (1938) has questioned the existence of genuine terminal deficiencies in Drosophila, on the grounds that chromosomes with such deficiencies would lack a stabilizing telomere structure.

The simplest explanation of the chromosome ends described in this paper is, however, that they originated by loss of the whole distal portion of the X chromosome, and that the broken ends healed and became functionally normal. There is no evidence to contradict this explanation. On this view, stable ends can be formed de novo from broken ends without attachment of a previously existing telomere. There seems no reason to doubt this

interpretation of these and other apparent terminal deficiencies in Drosophila, as well as the results of McCLINTOCK in maize.

SUMMARY

Five apparent terminal deficiencies in the chromosomes of *D. melanogaster* are described. From genetical and cytological data it is concluded that these are genuine terminal deficiencies, in which the normal chromosome end has been lost and a new end has been formed at the breakage point. This conclusion is incompatible with the assumption that existing chromosome ends, or "telomeres," are essentially permanent and indispensable structures.

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ANALYSIS OF WILD AMERICAN RACES OF OENOTHERA (ONAGRA)[1]

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THERE was a time when the evening primrose was the despair of the geneticist. It still is the despair of the taxonomist, who finds it difficult to classify and delimit species in this genus on the basis of phenotypic criteria alone. This difficulty is now known to be due to the fact that most Onagras are, from the phylogenetic point of view, not single, but dual entities. They are usually composites of two distinct, separable, and, as a rule, phylogenetically diverse components or genoms known as the RENNER complexes, one of which may largely dominate over the other phenotypically. These complexes are continuing entities of a more or less permanent nature, being passed intact from generation to generation in selfed lines.

The peculiar cytogenetic behavior of Onagra, which is responsible for this duality, is now familiar to all geneticists and will not be discussed here. The fact should be emphasized, however, that the genoms which are associated to form a given species are, in most races, very different from each other genetically, and probably of diverse phylogenetic origin. Thus, in tracing the affinities of a given race, one must consider two distinct lines of phylogenetic descent. The relationships of both genoms must be taken into consideration.

This is not an easy thing for the taxonomist to do, however, since one genom, by reason of its dominant genes, often effectively masks the other. Furthermore, races composed of two diverse genoms breed as true as do those whose genoms are essentially similar, thanks to their balanced lethals. Hence a taxonomist cannot tell by observation, or even by inbreeding, which races contain genoms of diverse phylogenetic origin and which contain genoms which are closely related. This can only be determined by cytogenetic means.

One example will show the necessity of the cytogenetic approach in tracing the phylogenetic position of a race in Onagra. "*Iowa 1*" is a race of the *biennis* type. It has broad, dark green crinkly leaves, small flowers and other so-called "*biennis*" characters, and it breeds true to type. So far as one can see phenotypically, it is *biennis* and nothing else. If one crosses it, as egg parent, to the *hookeri* of DEVRIES, the F_1 hybrids resemble "*Iowa 1*" very closely. But if one crosses it as pollen parent to *hookeri*, a very different sort of hybrid results, one which looks in no respect like a *biennis*, which differs from its reciprocal in almost every external character. Both

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hybrids have received the same genom from the homozygous *hookeri;* the differences between the two must therefore be due solely to "*Iowa 1*" itself, which, it turns out, carries a genom very different from the one which is able, by reason of numerous dominant genes, to control the phenotype. One would never have suspected, on looking at the *biennis* type, "*Iowa 1*," that it was carrying a genom capable of producing a plant so different from itself. Yet it is obvious that it is just as important to consider the affinities of this complex as of the other in determining the phylogenetic position of "*Iowa 1*."

This is not an isolated case. It is typical of the genus as a whole. What is true of "*Iowa 1*" is true of most of the wild races east of the Rocky Mountains. It at once becomes apparent, therefore, that a proper understanding of relationships in Onagra can be reached only through the determination of relationships between genoms. If we understand the phylogenetic position of the genoms of a given race, then we can understand the position of the race itself. This can, however, only be accomplished by cytogenetic means, for only by such means can one ascertain, first, whether a race is made up of two diverse genoms, and secondly what are the relationships between the genoms found in the various wild races.

We may now ask how it is possible, by cytogenetic means, to determine relationships between various genoms. This can be done to some extent by analyzing each genom genetically and comparing the genoms thus analyzed, a type of study made noteworthy by the work of RENNER. It is obvious, however, that a method necessarily so slow cannot be used in an attempt to determine relationships throughout the entire genus. Another method, however, which promises to make such an analysis possible depends upon the fact that relatively frequent reciprocal translocations in Onagra have resulted in many diverse arrangements or groupings of the end segments of the chromosomes. Each genom has its own characteristic segmental arrangement. When two complexes with diverse arrangements of segments unite to form a plant, the pairing of homologous segments results in the formation of chains instead of pairs. By studying the degree to which a given complex produces chains or pairs with certain standard complexes whose segmental arrangements are known, it is possible to analyze the segmental arrangement of this complex in terms of these standards and to determine to what degree and in what manner this complex bears a resemblance to other complexes from the standpoint of segmental arrangement.

It has been suggested in earlier papers (1931, 1935, 1937) that similarity in segmental arrangement is likely to be an indication of close phylogenetic relationship. Complexes which have similar segmental arrangements are likely to be closely related. This gives us a simple and unique tool with

which to study relationships in Oenothera. One has but to cross the various races, determine which complexes have similar arrangements of segments, and thus obtain excellent clues as to their phylogenetic relationships. It may be added, however, that this method, which is capable of tracing to some extent the record of the interchanges which have occurred in the evolution of a complex, is not so capable of ascertaining the sources whence its genes have come. Without entering into a discussion of this matter at present, it may merely be said that it is possible for a complex to acquire genes in more than one way from an associated and unrelated complex without the alteration of its segmental arrangement.

For several years the writer has been working, with the collaboration of Dr. P. A. Munz, of Pomona College, upon an analysis of the relationships between the various wild races of Oenothera. This work has been supported for five years by the American Philosophical Society and is now being assisted by the Rockefeller Foundation. It is our plan and hope to make first a broad survey in order to gain a picture of the general situation, after which more detailed studies of individual groups of races and of geographical areas will serve to make clear the details. As yet, we have gained only a rough idea of the situation, but we have gone far enough to feel certain that our method, if pursued far enough, is capable of yielding a clearer and more precise picture of evolutionary trends and relationships than has been hitherto obtained in this and perhaps in any genus.

We have, so far, grown and determined the chromosome configuration of about 140 races (not counting European races) and have studied hybrids involving 96 of these. In the case of some races one or both complexes have been completely analyzed; in the case of other races only a bare beginning has been made or the analysis is in one stage or another of completion. Out of this mass of information obtained from our crosses, details of which will be presented elsewhere, we are beginning to understand the relationships of some races or groups of races, but of others we know as yet little or nothing.

The groups of races which give evidence of having distinctive cytogenetic behavior will now be mentioned.

The hookeri alliance. This group of races is centered chiefly in the far southwestern portion of the United States and in northern Mexico. In all, 36 races have been examined. The group is characterized by large flowers, open pollination, paired chromosomes or small circles in diakinesis, and the absence of lethal factors. The commonest condition is the all-pairing condition; circles of four are not infrequent, but larger circles are rare. Circles involving more than eight chromosomes are unknown. The presence of numerous pairs or of small circles means, on the basis of our assumption, that the genoms making up any individual race of the *hookeri* type are

closely related to each other in segmental arrangement, and probably, therefore, are phylogenetically closely related.

When various races of *hookeri* are crossed, the hybrids between them also show mostly paired chromosomes or circles of four. Larger circles have rarely been observed. It is clear from this that genoms in different *hookeri* races are as closely related on the average as are genoms within individual races. In general, the conclusion seems unavoidable that the *hookeri* alliance is made up of a variety of races, all of which, however, are closely related to each other, as is indicated by the fact that their genoms are similar or identical in segmental arrangement.

Of the various segmental arrangements present in the races of *hookeri* which we have studied, one is much more commonly found than any of the others. This arrangement is written, in terms of the originally chosen standard (*hookeri* of deVries) 1·2 3·4 5·6 7·10 9·8 11·12 13·14. There is reason for considering this arrangement either ancestral to all others in Onagra or, at least, the oldest arrangement now extant in California.

The second group of races with distinctive cytogenetic behavior is the *irrigua* alliance. In New Mexico, and possibly in contiguous areas, a type exists known as *irrigua*. It resembles the *hookeris* in being large-flowered, open-pollinated, and alethal, but it possesses certain fairly distinctive phenotypic traits which have been supposed to set it more or less apart. I have examined some seven races of this type and find them to be composed for the most part of individuals having chromosome configurations[2] of intermediate character, such as a ⊙6 or a ⊙4, ⊙6 or a ⊙8. Because of the absence of lethals, however, these plants do not breed true to their configurations. A plant with a circle of four, a circle of six, for instance, will throw, in addition to the parental type, individuals with a circle of four alone, a circle of six alone, or seven pairs. Circles are maintained in the population, however, because open-pollination encourages crossing between individuals having genoms with different segmental arrangements.

When the *irriguas* are crossed with the *hookeris*, hybrids with intermediate configurations predominate, such as ⊙8, or a ⊙4, ⊙6. Since intermediate configurations such as these represent minimum differences between the complexes of, in most cases, two or three interchanges, the genoms of the *irriguas* give evidence of fairly close relationship both to the genoms of the *hookeris* and to those with which they are normally associated. In short, the *irriguas* have a variety of segmental arrangements which differ in moderate degree from each other and at the same time also differ in moderate degree from the *hookeri* arrangements.

The *irriguas* are of particular interest in the fact that they are almost the only races of Onagra so far found in the wild whose chromosome con-

[2] ⊙ signifies "circle of."

figurations are intermediate between the all-pairing condition and the presence of large circles. It is a curious fact that races with intermediate configurations are almost entirely confined (so far as our work shows) to New Mexico and adjacent areas. Certain strains of *longissima* from Utah, of *Jamesii* from Oklahoma, and of *hookeri* from New Mexico and Colorado have shared with *irrigua* this peculiarity. It is no doubt a significant fact that intermediate configurations should be found primarily in a region intermediate between the western area, in which the all-pairing condition predominates, and the eastern area, in which a circle of 14 is the all but universal configuration. It looks as though these forms may represent transitional stages in the development of one condition (probably the eastern) from the other (probably the western).

We come now to a third group possessing distinctive cytogenetic behavior. Plants of the *Rydbergii-strigosa* assemblage are found in the eastern and northern Rocky Mountain area and across the great plains, perhaps also as far west as the state of Washington. These plants, of which the chromosome configurations of 21 races have been examined, have shown without exception a circle of 14. All so far analyzed are true-breeding complex-heterozygotes, with balanced lethals and small or moderately small self-pollinated flowers. In these respects, they resemble races in the eastern part of the range. They have one distinctive cytogenetic feature in common with *irrigua*, however, namely the fact that both complexes in a given race (so far as analyzed) are fairly closely related to the *hookeri* complexes, as shown by the fact that they give chromosome configurations of intermediate nature when combined with the latter. In the *strigosa-Rydbergii* group, however, the two complexes making up a race are less closely related to each other segmentally than they are to the presumed common *hookeri*-like ancestor, whereas in *irrigua* they are about as closely related to each other as they are to the ancestral complex. In the one case, the complexes in a race represent the result of divergence in radically different directions; in the other case they have resulted from divergence in more or less similar directions. In spite of the marked differences in chromosome configuration as between the *irriguas* and the *strigosa-Rydbergii* group, however, these two groups are no doubt related in about the same degree to the *hookeris*. In different ways, both groups show phenotypic resemblance to the *hookeris*, also.

So far as our studies show, therefore, it may be said that the *irriguas* and the *strigosa* and *Rydbergii* forms have each their own distinguishing cytogenetic characteristics which may prove of definite diagnostic value. They both show on the basis of segmental resemblance a fairly close affinity to the *hookeris*, in the case of both complexes.

Another group of races that may be mentioned is the *Jamesiis*. These

differ among themselves in many ways but are all large plants with flowers about ten centimeters across, having extremely long hypanthia. Two races from Oklahoma have shown chromosome configurations of an intermediate nature, like the *irriguas*, and one of these, crossed with various *hookeri* complexes, gives hybrids with intermediate configurations. These are probably related fairly closely to the *hookeris*. A third race from Mexico has seven pairs. It is probably, if anything, more closely allied to the *hookeris* than are the others, although this is still to be tested cytologically.

We shall now turn our attention to the more easterly portion of the range. We find that practically all races east of the great plains have a circle of 14. A total of 47 such races has been examined; of these one has shown all-pairing chromosomes; all but four of the remaining 46 races have shown a circle of 14. All forms with a circle of 14 so far tested are permanent heterozygotes, breeding true because of balanced lethals. All are self-pollinating, and flower size ranges from small to intermediate.

These races have been sent me under a variety of names or under no name at all. They represent a great variety of types, classification of which on purely phenotypic grounds is extremely difficult. When cytological work was begun on these forms it at first looked as though the cytological situation would prove as hopelessly tangled and complex as the phenotypic situation. Every complex seemed to be different and distant from every other one. During the past year, however, evidence has begun to come in which gives us reason to hope that the situation will not prove to be beyond analysis. We are beginning to recognize certain great groups of races which are not only phenotypically similar, but which are characterized by the possession of certain distinctive associations of complexes. As yet this evidence is meagre and the outlines of the picture are vague and incomplete, but we now have hopes of ultimately analyzing them, as will be seen from the following.

During the past two years, Dr. B. L. HAMMOND, my assistant, has been specializing upon forms which fall under the name of "*biennis*" in the manuals. He finds that these, so far as examined, have a distinctive situation with regard to their complexes. They have one complex (usually the α or egg complex) which has a segmental arrangement closely allied to the *hookeri* arrangements and another (usually the β or pollen complex) whose segmental arrangement shows much less resemblance to the *hookeri* complexes. The former complex is responsible for most of the "*biennis*" characters, for example, broad, crinkly leaves; the other produces narrow foliage, and in general an effect entirely different from that seen in *biennis*. The specific segmental arrangements may vary from race to race, but in four races, three from various points in Kentucky (a middle western state), the fourth from Maryland, the α complexes are identical in segmental ar-

rangement, and the β complexes are also identical. Further study will show whether this cytogenetic situation is characteristic of all *biennis* forms. It has been found so far in 10 races, and several additional strains which have been only partially studied seem to be showing the same situation. We are inclined to expect, therefore, that the presence of a broad-leaved complex closely related to the *hookeri* complexes in segmental arrangement, and a narrow-leaved complex unrelated to the *hookeris* may prove to be characteristic of all forms of this (the *biennis*) group.

It may be added that the behavior of the so-called "gamete lethals" varies from race to race within the *biennis* group. Some of these races are strictly heterogamous, some are isogamous, and some show intermediate conditions. There are one or two in which the broad-leaved complex is the pollen (β) complex, and the narrow-leaved one is the egg (α) complex. In every case, so far, however, it is the broad-leaved complex which is related segmentally to the *hookeris*.

Another group which shows indications of having a characteristic association of complexes is the *grandiflora* group, of which my strains have all come from the southern coastal plain (Alabama, Mississippi, and Florida). This group has thus far shown a similar situation to the *biennis* group, in that one complex is closely allied segmentally to the *hookeris*, the other is unrelated. The complexes of the *grandifloras*, however, produce phenotypic effects quite different in many particulars from the *biennis* complexes and have perhaps developed quite independently of the latter. I have not gone deeply enough into this group to say more at present.

A third group which seems to belong to *nutans* and *laevigata* has been investigated in a preliminary way only, and the results are not entirely clear. Indications are that both complexes in these forms produce broad, fairly crinkled leaves and slender buds, but that one complex produces these effects more strikingly than the other. Early returns from the cytological investigations suggest that both complexes may bear a fairly close relationship to the *hookeris*. There has not been time as yet to compare them with the *biennis* and *grandiflora* complexes.

This, in barest outline, is the general situation as far as it is known at present. Much work remains to be done on the groups mentioned, and several other groups await investigation. It seems clear, so far, that the *hookeris*, the *irriguas*, the *Rydbergiis* and *strigosas* and possibly the *biennis* and *grandiflora* groups have their own characteristic types of complex from the standpoint of segmental arrangement. It is perhaps not too much to expect that other groups will also show distinctive features.

We may now ask how the situation found throughout most of the range of Onagra is to be interpreted. How has the union of diverse complexes into a plant possessing a chain of 14 chromosomes come about, and what

is the reason for the survival, with few exceptions, of only those plants with diverse complexes and a circle of 14? In this connection, one fact seems to stand out as of particular importance, namely, that the complexes within a race often show less affinity for each other segmentally and genetically than they do for certain complexes in other races; this strongly suggests that hybridization has played an important role in the production of the various races. Through natural crossing, diverse complexes have been brought together. Many of these hybrids have bred true, owing to the lethals present, and have become established as true-breeding races or species. We are confronted, therefore, with the possibility that a large percentage of the Onagra population is actually of hybrid origin.

But we know from hybridization experiments that there is at least an equal chance that crossing will produce hybrids with some other configuration than a circle of 14. Why have only those with a circle of 14, with rare exceptions, survived? The answer is perhaps to be found in the suggestion (cf. DARLINGTON, 1929) that this configuration has survival value because it maintains a maximum of heterozygosity with a minimum of resultant sterility. We may state the case in this way: self-pollination is theoretically the surest and most efficient method of ensuring the setting of seed, even in large-flowered forms, but especially in those which come to have small flowers. It has, however, the disadvantage of reducing heterozygosity and thus depriving the plant of the benefits of hybrid vigor. Furthermore, as long as the chromosomes remain paired, balanced lethals are of little value, in fact are disadvantageous because they cut down fertility. Balanced lethals in a single chromosome pair would preserve the heterozygosity of this one pair alone, and would at the same time cut normal fertility in two. If additional chromosome pairs had balanced lethals, the proportion of genes preserved in the heterozygous condition would be correspondingly increased, but half of the remaining fertility would be lost for every additional chromosome pair having lethals. Thus, if all seven pairs in an all-pairing Onagra had balanced lethals, fertility would theoretically be reduced to less than 1 percent. Should, however, all or most of the chromosomes come to be linked into one chain, a single pair of lethals would preserve the heterozygosity of the whole or nearly the whole set of genes, with only 50 percent sterility. With heterozygosity of all or practically all the chromosomes thus preserved, cross-pollination would no longer be of value, and the increased certainty of seed setting resulting from self-pollination would probably more than make up for the sterility due to the lethals.

Hence it is evident that the presence of a circle of 14 has come to have a unique survival value. It has given to the plants possessing it an advantage in the struggle for existence, for it has permitted the setting of a

plentiful supply of seed and at the same time has ensured to the plant hybrid vigor in a greater degree than is available to plants which have fewer chromosomes covered by balanced lethals. Hybrids with other configurations than a circle of 14 have therefore been largely weeded out. We see also why it is that balanced lethals have been found only in races with large circles.

In this brief statement it has been possible only roughly to indicate the course of our studies and to suggest the nature of the results which are being obtained. Detailed analyses of segmental arrangements in the various complexes and a full discussion of the results of these analyses will be presented in succeeding papers.

SUMMARY

Certain cytogenetically rather well-defined groups of races are now becoming recognized which may be called tentatively by the names *hookeri*, *irrigua*, *strigosa*, *biennis*, etc. In a given race of *hookeri*, associated complexes are identical or similar in segmental arrangement, and this is true also of complexes in different races of *hookeri*. In the *irriguas*, associated genoms are more or less similar, frequently giving chromosome configurations which include one or more small or medium-sized circles. These genoms show the same degree of relationship in segmental arrangement to the *hookeri* genoms that they do to each other. In the *strigosas*, associated complexes are quite unlike in segmental arrangement, giving a circle of 14, but both complexes in a given strain are in this regard rather closely related to the *hookeris*. In each of the *biennis* forms so far studied, the complex producing broad leaves is closely related in segmental arrangement to the *hookeri* complexes; the opposing complex, which produces narrow leaves, is less closely related. Other groups, which are less thoroughly studied, seem also to show characteristic features. Facts such as these, when they become more numerous, should help us to discover such natural groupings as exist in the Onagras and to understand how these various groups are related to one another.

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THE TIME OF EFFECT OF SOME SECOND CHROMOSOME LETHALS IN DROSOPHILA MELANOGASTER

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THE purpose of this study was to determine which of a series of lethals are effective in the egg stage.

Six different lethals, carried as Curly balanced stocks, were obtained from Dr. PLOUGH of Amherst College. Each of these lethals, numbered 1, 4, 9, 11, 19, 21, was isolated from wild Belfast-Maine stock in the fall of 1938. The lethals are not associated with inversions or translocations; it has not yet been determined if they are associated with deficiencies. They are all located in the second chromosome.

In a later paper experimental methods will be described in some detail. For the collection of eggs the techniques described by the writer (1939) were used. Where later stages of development (that is, larval and pupal) were not to be studied, it sufficed to align the eggs collected at the end of twenty-four hour intervals in rows of ten on the surface of the medium used for egg collection. When later stages were to be followed, eggs were transferred to strips of black photographic paper lying on the surface of the medium in Petri dishes and again aligned in rows of ten. In both cases, counts were made after twenty-four hours and again after forty-eight hours to determine embryonic mortality. The medium was liberally supplied with yeast so that lack of food would not be a factor in larval mortality. All work was carried out at 25°C.

EXPERIMENTAL DATA

For the data in table 1, the eggs laid by $l/+ \times l/+$ (outcrossed with Canton +) were counted first to eliminate mortality possibly due to the "Curly" lethal. Canton+ egg counts were used as controls to determine "normal" embryonic mortality (residual mortality). MSC, an Amherst wild strain, mass cultured for two years, was also tested. No significant difference was found for embryonic mortality in a comparison between counts of sib matings, $l/+ \times l/+$, and Cy/l counts from stock matings. Table 1 lists the data on embryonic mortality of the six lethals from the Belfast-Maine stock in addition to that of the lethal associated with the *Cy* inversion and of the controls.

Disregarding the residual mortality only l_{21} of the six lethals from the Belfast-Maine stock showed any indication of being effective wholly in the egg stage. The data in table 2 were collected to determine whether any of the embryonic mortality could be ascribed to the lethal associated with the Curly balancer. Table 2 shows the mortality for three developmental

stages of the offspring of the Cy/l_{21} stock, Cy/Canton+ × Cy/Canton+, and Canton+ stock (for an estimate of residual mortality).

TABLE 1

Mortality of different lethals and controls in the embryonic stage.

PARENT GENOTYPE	TOTAL NO. OF EGGS	UNHATCHED EGGS	PERCENT EGG MORTALITY
$l_1/+$	180	24	13.3±2.6*
Cy/l_1	143	24	16.8±3.1
$l_4/+$	319	56	17.5±2.1
Cy/l_4	227	45	19.8±2.6
$l_9/+$	273	62	22.5±2.5
Cy/l_9	350	74	21.2±2.2
$l_{11}/+$	378	49	13.0±1.7
Cy/l_{11}	191	24	12.5±2.4
$l_{19}/+$	362	14	3.9±1.0
Cy/l_{19}	306	26	8.5±1.6
$l_{21}/+$	321	76	23.7±2.4
Cy/l_{21}	1882	492	26.1±1.1
Canton+	595	35	5.9±0.9
MSC+	804	69	8.6±1.0
$Cy/+$**	471	37	7.9±1.2

* Standard error of a percentage.
** Outcrossed with Canton+.

TABLE 2

	Cy/l_{21}	$Cy/+ \times Cy/+$	CANTON+ CONTROLS
eggs observed	1036	471	595
unhatched eggs	261	37	35
percent embryonic mortality	25.2±1.3	7.9±1.2	5.9±0.9*
larvae observed	547	121	500
larvae dead	168	30	33
percent larval mortality	30.7±2.8	24.8±4.0	6.6±1.1
corrected mortality	22.9±1.8	22.9±3.7	6.2±1.1
pupae observed	160	98	213
pupae dead	12	7	5
percent pupal mortality	7.5±1.8	7.2±2.5	1.9±0.9
corrected mortality	3.9±1.5	5.0±2.2	1.7±0.9
Total mortality (corr.)	**52.1%**	**35.7%**	**13.7%**

* Standard error of a percentage.

While the data given above indicate the embryonic mortality of the lethal associated with the particular Curly balancer used, they do not

throw any light on the problem of residual mortality. Residual mortality is the product of two factors, genetic and environmental. Since the environmental factor is presumably uniform in all of the above counts, there remains only the genetic variability of the material to be accounted for in the above data. The frequency of lethals, or possibly the presence of modifying factors in the other chromosomes, determines how much of the residual mortality is due to the genetic condition of the material. The extraneous lethals may be eliminated by inbreeding the balanced stock, but this does not remove the possible influence of modifying factors. Accordingly, Cy/l_{21} females were outcrossed with Canton+ males and with

TABLE 3

GENOTYPE OF PARENTS*	1	2	3	4	5
eggs observed	667	302	477	214	428
unhatched eggs	113	55	22	13	6
percent mortality	16.9±1.5	18.2±2.2	4.6±0.9	6.1±1.4	1.4±0.5
larvae observed	332	247	455	201	206
larvae dead	26	20	111	51	3
percent larval mortality	7.6	8.1	24.4	25.4	1.5
corrected mortality	6.5±1.2	6.6±1.4	23.2±2.0	23.4±2.9	1.4±0.7
pupae observed	306	227	444	150	203
pupae dead	18	13	4	1	2
percent pupal mortality	5.9	5.7	0.9	0.7	1.0
corrected mortality	4.5±1.0	4.3±1.2	0.8±0.13	0.5±0.1	0.9±0.3
Total (corrected)	**27.9±2.2**	**29.1±2.6**	**28.6±2.1**	**30.0±3.1**	**3.7±1.3**

* Legend: 1. l_{21}/Canton ♀♀ × l_{21}/MSC ♂♂
2. l_{21}/Canton ♂♂ × l_{21}/MSC ♀♀
3. *Cy*/Canton ♀♀ × *Cy*/MSC ♂♂
4. *Cy*/Canton ♂♂ × *Cy*/MSC ♀♀
5. l_{21}/Canton × *Cy*/Canton

MSC males. Wild type flies of the two F_1's were then crossed with each other and counts were made of the developmental stages of the "synthesized" F_2. The purpose of such a procedure was to reduce as much as possible the genetic residual mortality by increasing heterozygosity in the other chromosomes. The results are tabulated in table 3. "Corrected" percentages are expressed on the basis of original number of eggs.

Some of the dead eggs were dechorionated and examined under the microscope. The eggs were classified as early or late depending on whether tracheal rudiments had been developed. These are formed by the eleventh to twelfth hour according to POULSON (1937). Complete data will be given in a later paper on developmental studies. For the present it suffices to say

that the majority of the wild eggs died before the appearance of tracheal rudiments, that is, were "earlies," while the eggs of both *Cy* and l_{21} were preponderantly "lates."

DISCUSSION

The theoretical expectation for mortality, that is, 50 percent for the balanced Cy/l_{21} stock and 25 percent for the lethal itself, is closely approximated in the data above, if all of the developmental stages are taken into consideration. If, however, we apply the specificity idea to the time of action of lethal genes, we should expect all of the mortality to occur in one of the developmental stages, that is, embryonic, larval, or pupal, for each lethal in question. That none of the lethals studied adheres closely to this rule is obvious from the data presented. While the data of table 1 would seem to indicate that as far as $l21$ is concerned, the theoretical expectation is adequately fulfilled ($l21$ expressing itself in the egg stage and *Cy* in the larval stage) the data of table 3 show otherwise. The apparent fulfillment of expectation would disregard residual mortality. Thus, the data of table 1 when corrected for residual mortality are comparable to that of table 3. In the case of $Cy/l21$ the embryonic mortality, when corrected for residual mortality, becomes 18.2 percent compared to 16.9 percent and 18.2 percent from table 3. While the data available for the later stages of $l19$ are insufficient for tabulation, it may be said that of the six lethals studied, it alone comes close to being stage specific, most of the mortality occurring in the larval stage.

This apparent lack of specificity has been evident in earlier reports on the time of effect of lethals (DEMEREC and HOOVER (1936), BREHME (1937), KALISS (1939), and others). On the other hand, many other lethals seem to be specific as reviewed by STERN (1939). The data given above for the time of effect of the lethal associated with the particular Curly inversion used as balancer in this study agree with those of SIVERTZEV-DOBZHANSKY (1927). Although she interpreted the data as indicating that Curly itself was the lethal, she found that death occurred chiefly in the second instar. Presumably the two stocks carry the same lethal. It will be noted that the figures obtained by the writer for Canton+mortality agree very well with those obtained by BREHME (1937) for Florida +. The problem of residual mortality has been attacked by many investigators, STURTEVANT and BEADLE (1936), SCHWEITZER and KALISS (1935), and others. In general it may be said that residual mortality varies from about three percent to about eight percent, depending probably more on the genetic composition of the stock than on environmental factors. The lower limit of residual mortality is obviously that set by mutation frequency, and it is therefore impossible to eliminate it entirely. The extreme environmental factors

which may increase residual mortality, and which are rarely encountered in a well conducted experiment, are dessication in the case of eggs not laid on the medium, and suffocation by overgrowth of yeast.

Several explanations, or rather speculations, may be offered for the apparent lack of specificity of time of action manifested by the above and other lethals. We may assume the presence of other lethals or modifying factors either in the chromosome studied or in the other chromosomes. While the technique of outcrossing to increase heterozygosity does not eliminate entirely this possibility, it reduces it considerably. The factors which would be most difficult to eliminate are those present on the same chromosome and closely linked to the lethal being studied. However, if we assume random distribution for such factors throughout the whole chromosome complement, their influence becomes negligible compared to the obtained results. There remains then one other explanation.

Heretofore, we have been separating arbitrarily the egg stage or so-called embryonic stage from the larval stage, while actually the newly eclosed larva is already in the first instar, that is to say, development up to the first ecdysis is one continuous process. We may suppose that development, at least as far as most larval organs (with the possible exception of those lost at each moult) and imaginal anlage or discs are concerned, is a continuous process through all of the "stages of development." This statement should perhaps be limited for the larval organs, where development is continuous at least until the pupal period, when they degenerate. This may perhaps explain the infrequency of pupal lethals. Now if we postulate that some lethals have a wider range of Gaussian variation than others, we may think of the "specific" time of action as being the modal time of action. Where there is a deficiency of some substance necessary for a transition from one stage to another, we may expect to find this curve of variation present up to the transition point and then abruptly broken off. Thus, for example, if the lytic enzyme necessary for the rupture of the vitelline membrane prior to eclosion is absent, or if a hormone (or its precursor, which is ultimately of genic origin) necessary for an ecdysis or pupation is absent, development in terms of "stages" terminates abruptly and we have an apparently specific time of action. The variation which precedes this event is normal in every respect. The eggs studied after dechorionation manifested this normality of variation to such an extent, that for the purposes of this study it was necessary to set up an arbitrary line of classification in the time scale (that is, eleventh to twelfth hour—see above, experimental data). Of those eggs which died later than the arbitrary time, none had mouth parts which had turned black, although many individuals had developed fully to pre-eclosion larvae and still showed signs of motion in wet mount preparations. It is suspected that one of the factors involved in this

lethality is an upset of the tyrosinase-oxidase system. Respiration as well as developmental studies have been begun.

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SUMMARY

1. Seven lethals were studied to observe their embryonic mortality. They showed different amounts of embryonic mortality varying from 2 percent below the wild type control to 19.3 percent above it.

2. The evidence seems to indicate that a lethal may manifest itself at any stage of development with no specific time of action at which all of the homozygotes of a particular lethal die. Attempts to eliminate extraneous factors which possibly bring about this condition of "non-specificity" show even more conclusively the existence of such "non-specificity."

3. Possible explanations for this apparent lack of specificity are given.

4. The lethal associated with the particular Curly inversion used as balancer in this study manifests itself chiefly in the second instar, and is apparently the same as that used by SIVERTZEV-DOBZHANSKY (1927) in her studies.

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INDEX TO VOLUME 25

Page numbers printed in **boldface** refer to headings and main citations.

aberration (See chromosomal abberrations)
abstracts, Genetics Society, 109–141
Achyranthes, 410
Acnida cuspidata, 410
Acnida tamariscina, 128, 410
Acnida tuberculata, 410
AGOL, I. J., 571
Agropyron cristatum, 129
albinism, 110
alfalfa, **1**–15
alfalfa endosperm, 611
ALLEN, E., 323
ALPATOV, W. W., 246
Amaranthaceae, sex determination, **409**–431
Amaranthus caudatus, 410
Amaranthus hybridus, 410
Amaranthus Powellii, 410
Amaranthus retroflexus, 410
Amaranthus spinosus, 410
American Philosophical Society, 638
Amherst College, 354
amixis in evolution, **109**
amphidiploids, 401
ANDERSON, EDGAR, 438, 439, 440, 442, 448, 449, 450, 453, 459, 462
ANDERSON, E. G., 150, **299**–309
antipodal cells, 595
aphid, 125
  intermediate winged, **134, 287**–298
aristapedia, **143**–149
Arnold Arboretum, 41, 134, 136
Arrhenantherum elatius, 129
ASMUNDSON, V. S., 35
ASTAUROFF, B. L., 149, 349
ATWOOD, S. S., **109**
autotetraploid grasses, **129**
AVERY, A. G., **111**, 310, 508, 509, 510

BABCOCK, E. B., 82
BACKHOUSE, W. O., 2
BALKASHINA, E. I., **143**–149
BANTA, A. M., **310**–325
BANTA, S. M., **109**
Bar, 126, 158
BARROWS, W. M., 246
BARTLETT, M. S., 164
BARTO, E., **110**
BATE, R. C., 43
BAUER, H., 47, 58, 61, 474, 475, 479
BAUR, E., 150, 154
BEADLE, G. W., 95, 146, 299, 300, 339, 376, 380, 381, 382, 383, 452, 455, 487
BEAMS, H. W., 485
BEATTY, A. V., **110**
BELAR, K., 57, 483
BELGOVSKY, 575, 576, 582
BELLING, J., 508
BERG, 575
BERGNER, A. D., **111**
BERNSTEIN, F., 187
biovular twins, **187**–196
BIRAGHI, A., 607
BLACK, W. G., 326
BLACKETT, P. M. S., 80, 310
BLAKESLEE, A. F., **111**, 133, 139, 285, 508, 509, 510, 596, 614
BLAIR, A. P., **110**
BLANC, R., **111**, 361
blood tests in twins, **187**–196
BOCHE, R. D., 61, 84
BODENSTEIN, D., 246, 584
BORISOFF, 575
BOYE, C. D., **132**
BOYES, J. W., 1, 11, 612, 614
Brachyury in mouse, 116
BRADBURY, DOROTHY, 614
BRANDLY, C. A., **139**
BRAUN, W., **111, 143**–149, 584
BREHME, K. S., **112**, 229, 585, 589, 590, 648
BREMER, G., 12
BRESSMAN, E. N., 421
BRIDGES, C. B., i–v, 149, 226, 239, 522, 536, 618, 622, 632, 633
BRINK, R. A., 1, 2, 7, 13, **113, 114, 299**–309, **593**–617
bristles, 143–149, **225**–250, **251**–277, **354**–365
bristle pattern in Drosophila, **251**–277
BRODY, G., **645**–650
Brown University, 109, 119, 127
BRYSON, V., **113**, 584, 589
BUCHHOLZ, J. T., 285
BUCK, J. B., 466
BURNHAM, C. R., 285, 303, 487

CAIRNS, J., 223
California Institute of Technology, 299, 337
Capsicum frutescens, 596
CARLSON, J. G., 63
Carnegie Institution of Washington, 111, 112, 115, 124, 127, 128, 133, 139, 534, 618, 628
CARTER, H. G., 433
CASPERSSON, T., 539
CASTLE, W. E., 158
CATCHESIDE, D. G., 58, 474
cell division, effects of colchicine upon, 116
cellular characters in Columbidae, 123, **326**–336
Celosia, 410
Central Experimental Farm, 126
centromere stability, **483**–520
centromere structure, 506
Chaetopsis fulvifrons, 35
CHAMBERLAIN, H. B., 178
CHARLES, D. R., 236, 275
CHEN, TSE-YIN, 591
cherry, sour, 614
CHESLEY, P., 391, 392, 393, 399
chiasma frequency, 449
CHILD, G. P., 245, 252, **354**–365, 584, 589, 590
chimerism, **16**–40
chondrodystrophy, 215
CHRISTIE, W., 17, 18
CHRISTOFF, M., 599
chromatic granules, **127**
chromatid breaks, 42, 76

chromosome
behavior in autotetraploid grasses, 129
bridge configurations, 72
chromocentral region, 565
deletions, **466**
heterochromatic region, 565
heterochromatin in Drosophila, **618**–650
inert region, 565
rye, 401
salivary, **534**–540, 618, 622
splitting, time of, 55
telocentric, **483**–520
X-rays, sensitivity to, 60, 117, **140**
chromosomal aberrations, iv, **41**–68, **69**–87, 117. 124, **129**, 132, 134, 299–309
Apoletlix eurycephalus, 129
Drosophila, 117
frequency of various types, 77
induced by X-rays, 41–68, 124, 134
dosage, 46, 51, 59
intermittent radiation, 52
inversion, **138**
loci, 46, 59
maize, 299–309
natural radiation, 78
spatial arrangement, 45–46
mutation rate, 132
spontaneous, 69–87, 117, **128**
Tradescantia, 41–68, 69–87, 132
chromosomal deficiency, iv
Chymomyza amoena, 351
Chymomyza procnemis, 351
Cladocera, 109, **310**–325
CLANCY, C. W., 339
CLARK, A. H. 246
CLARK, F. J., **136**, 389
CLARKE, A. E., 300
CLELAND, R. E., 636
coat color
guinea pig, **197**–214
mouse, 158
Coe College, 137
colchicine, 116, **310**–325
COLE, L. J., **16**–40, **123**, 326–336
Coleus blumei, **132**
COLLINS, G. N., 12, 99, 100
COLLINS, J. L., 82
color, flower, 133
Columba, **123**, **326**–336
Columba livia, **16**–40
Columbia University, 113, 114, 116, 136, 391,
COMBS, J. D., 246
Connecticut Agricultural Experiment Station, 136, 366
COOPER, D. C., 1, 3, 7, 12, 13, **113**, **114**, **593**–617
Cornell University, 88, 122, 128, 409
CORRENS, C., 150, 154, 409, 418
Cosmos bipinnatus, **150**–156
cotton, 121
COYNE, J., 584, 589
CRANE, M. B., 12, 150, 278, 285
Creeper fowl, **215**–224
CREW, F. A. E., 24, 34, 37
crossing over, ii, **130**, 452
CROZIER, W. J., 480
Cucurbita, 136
culture medium, Drosophila, 252
CUMLEY, R. W., **114**, **123**
CURRY, 522
CURTIS, M. R., **114**

Dactylis glomerata, 126, 129
DANFORTH, C. H., 24, 37
DANIEL, J., 197
Daphnia, 109, **310**–325
DARLINGTON, D. C., 12, 439, 443, 444, 445, 450, 451, 454, 455, 462, 483, 485, 486, 489, 490, 502, 504, 506, 507, 508, 509, 510
DARWIN, C., 17
Datura, **111**, 596
Datura metel, 614
Datura stramonium, **133**, 596
DAVIS, B. M., **433**–437
DAWSON, W. M., 115
deer mouse, **110**
deficiency
Drosophila, 521
maize, 299–309
DELBRÜCK, M., 82
deletions, **466**
DEMEREC, M., 47, 61, 81, 83, 115, 264, 388, 466, 474, 475, 478, 479, 480, 522, 534, **618**–627, 628, 629, 648
DERMEN, H., 310
development
embryonic, 128, 217
larval, 111, 143–149
Medicago embryo, 2–6
optic discs, 112
oxygen, effect on, **126**
temperature, effect on, 225–250
wing, 125
dicentric bridge formation, 634
Dichaete, 225–250, 546
DICK, J. R., 326
differentiation
of aphid types, 125
in chromosomes of Datura, 133
of gonads, **121**
diploidy, Medicago, 1
divisibility, gene, 541
DOBROVOLSKAIA-ZOWADSKAIA, N., 116, 391, 392
DOBZHANSKY, TH., 149, 157, 159, 160, 176, 337, 338, 340, 349, 351, 438, 454
dog, 115
dominance, house mouse, 116
dominant lethal, Drosophila, 137
DOMM, L. V., 31
DONALD, H. P., 346
DORSEY, M. J., 150
double fertilization in plants, 113
DOUGAN, P. K., 137
DOWNEY, J. E., 180
DRIVER, E., 354
DRIVER, O. W., 246, 354
Drosophila
bristles, 548
bristle patterns, 251–277
complementary genes for sterility, 562
duplications, 568
fertility, 561
inversions, 542, 544, 563, 579
Notch, 388
repeats, 568
sterility, complementary genes, 562
susceptibility of sexes to X-rays, 117
translocations, 563

virilis complex, 131
Drosophila affinis, 337–353
genes
abdomen rotatum, 344
abnormal, 340
antennipedia, 342
arc, 340
ascute, 339
bithorax, 340
bobbed, 338
cinnabar, 342
claret, 340
crossveinless, 341
crumpled-1, 341
crumpled-2, 341
cut, 339
Delta, 341
Fused, 344
hairy, 339
hairy-2, 340
jaunty-1, 342
jaunty-2, 342
jaunty-3, 343
knot, 341
miniature, 343
narrow, 342
net, 343
pauciseta, 341
pebbled, 339
pinkish, 341
pink-like, 342
reduced, 344
roughish, 342
rugose, 341
scarlet, 340
scute, 343
scute-1, 339
scute-2, 339
sex-ratio, 340
short-1, 341
short-2, 341
short-tarsi, 341
short-wing, 341
straw, 342
stubby, 339
tilt, 340
tiny-bristle, 341
truncate, 344
veinlet, 340
vermilion, 339
white, 343
white-1, 339
white-2, 339
yellow, 343
Drosophila algonquin, 347
Drosophila ananassae, 337, 634
Drosophila athabasca, 337
Drosophila auraria, 347
Drosophila azteca, 337
Drosophila bipectinata, 347
Drosophila bromeliae, 351
Drosophila cardini, 351
Drosophila coracina, 351
Drosophila duncani, 351
Drosophila earlei, 337
Drosophila ficusphila, 348
Drosophila florae, 351
Drosophila funebris
bristle pattern, 251–277
Polychaeta, 256
Drosophila iroquois, 337
Drosophila lutea, 348
Drosophila melanogaster, 337, 618, 628
bar, 126
bristle formation, 354–365
bristle pattern, 251–277
centromere, 624
character expression, 225–250
corresponding mutant types, 343
crossing over, 130
deficiencies, 619, **628**–635
deficiency, temperature effects, 521
development, 225–250
differentiation of gonads, 121
duplication, 585, 628
duplications, viability of, 626
euchromatic insertions, **618**–650
genes
abdomen rotatum, 343
achaete, 572, 628
apl, 546
apricot, 544
ascute, 343
bar, 126, 544, 584
bifid, **138**, 618
bithorax, 343
bobbed, 343
bobbed lethal, 628
brown, 628
carmine, 618
carnation, 544, 628
cinnabar, 343
claret, 343, 546
crossveinless, 618
crossveinless-d, 343
crumpled, 343
Curly, 546, 645
curlex, 618
cut, 343, 618
Delta, 343
deltex, 618
diminutive, 618
dumpy, 343
ebony, 628
echinus, 618
eyeless, 628
facet, 618
forked, 628
garnet, 618
hairless, 343
hairy, 343, 546
hairy wing, 628
infrabar, 126
jaunty, 343
lanceolate, 343
Lobe, 584
lozenge, 544
miniature, 343, 544
Minute, 112, 584
Minute-4, 628
net, 343
Notch, 618, 628
pebbled, 618
pink, 343
prune, 618, 628
roughest, 618
roughex, 618
roughoid, 546

ruby, 618
rucuca, 546
rugose, 618
scarlet, 343
scooped, 618
scute, 343, 547, 618, 628
scute-1, 354
shaven-naked, 628
shifted, 618
singed, 618
speck, 546
split, 628
straw, 343
tilt, 343
veinlet, 343
vermilion, 343
vesiculated, 618
white, 343, 544, 618, 628
yellow, 343, 544, 572, 618, 628
heterochromatin, **618**
induction of chromosome changes, 124
infrabar, 126
insertional translocation, 618
inversions, 549
larval development, 111
life cycle, 585
map, v
Minute, 112
mortality, 648
mottling, 623, 625
mutations, 584
aristapedia, 143–149
bifid, 138
dachs, 144
Dichaete, 225–250
hairy wing, 264–267
polychaetoid, 225–250, 259–264
thickoid, 144
vestigial, 119
wrinkled, 138
oxygen, effect on development, 126
phenocopies and X-radiation, 111
salivary glands, 137
salivary gland chromosomes, 534–540
second chromosome lethals, 645
spermatheca shape, 157–177
temperature, effect on vestigial, 119
terminal deficiencies, 628
translocation, 628
transplantation, 112, 143
transposition, 618
vestigial, 584–592
wing development, 120, 138
wing mutants, **584**–592
Drosophila miki, 347
Drosophila miranda, 347
Drosophila montium, 347
Drosophila mulleri, 351
Drosophila narragansett, 337
Drosophila nebulosa, 347
Drosophila obscura, 337
Drosophila phalerata, 351
Drosophila pseudoobscura, 337
corresponding mutant types, 343
Drosophila quinaria, 351
Drosophila ramsdeni, 351
Drosophila robusta, 351
Drosophila rufa, 348
Drosophila saltans, 347
Drosophila seminole, 337
Drosophila similis, 351
Drosophila simulans, 338
Drosophila subobscura, 337, 350
Drosophila suzukii, 348
Drosophila takahashii, 347
Drosophila transversa, 351
Drosophila virilis, 351
Drosophila virilis americana, 351
Drosophila willistoni, 337
DUBININ, N. P., 252, 541, 571, 575
DUNHAM, H. H., 310–325
DUNN, L. C., **116**, 197, 215, 391, 392, 393, 584, 589, 590
DUNNING, W. F., **114**
duplication, v
DURHAM, F. M., 197

ear differences in Zea-Euchlaena hybrids, 88, 102
EAST, E. M., 11, 150, 152, 153, 154, 599
EATON, O. N., 35
EBERHARDT, K., 469
EIGENBRODT, H. J., 236, 246
EIGSTI, O. J., 116, 310
EINSELE, W., 197
ELLENGORN, J., 572
ELLERTON, S., 444, 451, 454
embryo development
Creeper fowl, 217
differentiation of gonads, 121
Medicago, 2–6
Sciara, **128**
EMERSON, R. A., 88, 95, 285, 299, 300, 376, 380, 381, 382, 383, 409, 410, 414, 429, 487
Emory University, 131
emotionality in rat, **130**
EMSWELLER, S. L., 72, 73, 84
endosperm, 593
ENZMANN, E. V., 476, 480
EPHRUSSI, B., 146
Erythronium americanum, 131
Eschscholtzia, **110**
evolution, **109**

FABERGÉ, A. C., 67, 472, 477
FELDMAN, H. W., 18, 158
FERGUSON, M. C., 596
fertilization
double, 594
in plants, 113
FILZER, P., 150, 154
fish, gene frequencies in, 118
flecking in pigeon, 16–40
birds, 20
Floradale Farms, 150
FLORELL, V. H., 402, 405
flower color, **133**
flower form, **140**
fowl
Creeper, **215**–224
linkage in, 122
viability, **139**
FRANDSEN, G. G., 300
FRANKEL, O. H., 459
FRASER, A. C., 299, 300, 376, 380, 381, 382, 383, 487
FRIEDENREICH, V., 193
FROLOWA, S. L., 349
FRYER, J. R., 1

fusion, **124**
FYODOROVA, 546

GAIRDNER, A. E., 11, 454
Galton and Mendel Memorial Fund, 409, 593
gametic lethals, **366**–390
GARDINER, E. J., 584
GÄRTNER, C. F., 599
GAUSE, G. F., 246
gene
  divisibility, 541, 570
  frequencies in fresh-water fishes, 118
  number and size, 570
Genetics Society Abstracts, 109–141
geographic relationships among pigeon species, 114
GERASSIMOVA, H., 83
GERSHENSON, S. M., 565
GETTING, I. A., 85
GHIGI, A., 17
GILES, N., 41, 63, **69**–87, **117**, 455, 459, 462, 467, 473
GLASER, O., 358
GLASS, H. B., **117, 118**
GLIŠIČ, L., 596
GLUECKSOHN-SCHOENHEIMER, S., **391**–400
GOLDEN, A., 326
GOLDSCHMIDT, R., 143, 149, 252, 270, 287, 360, 576, 584, 591
Gomphrena, 410
gonads, 121
GONZALEZ, B. M., 157
GOODSELL, S. F., 478
GOODSPEED, T. H., 65, 467, 508
GORDON, M., **118**
Goucher College, 117, 118
GOWEN, J. W., 118, **140**
GREEN, C. V., 158
GREEN, E. L., **119**
GREEN, M. M., **119, 584**–592
GREITE, W., 24
GRIFFEN, A. B., **131**, 485
GRIMBALL, P. C., 409
GUIGNARD, L., 596
guinea pig, coat color, **197**–214
gynandromorphism, iii, **16**–40

HAERTL, E. J., **124**
hairlessness in deer mouse, 110
HÅKANSSON, A., 510
HALDANE, J. B. S., 157, 164, 176
HAMBURGER, V., **215**–224
handedness, **178**–186
HANSON, F. B., 82, 575
HARNLY, M. H., **120**, 246, 354, **521**–533, 590
HARNLY, MARIE L., 521, 524, 526, 527, 529, 530, 532
HARTWELL, G. A., 212
Harvard University, 41, 69, 117, 124, 132, 134, 138. 438, 466, 645
HAWKINS, L. E., 16, 17
HAYDAK, M., 212
HAYES, H. K., 599
HEFNER, R. A., **120**
HEIDENTHAL, G., **197**–214
hermaphroditism, 151
HERSH, A. H., 158, 246
HERTWIG, P., 20, 22
heterochromatin in Drosophila, **618**–650
HEYS, F., 82
HILL, H. D., **129**
HOFMEYR, J. D. J., 409
HOLLANDER, W. F., **16**–40
HOLLINGSHED, L., 11
HOLMES, W. F., 36
homoeosis, 143
HOOVER, M. E., 264, 388, 455, 462, 628, 629, 648
HORLACHER, W. R., 30
HUDSON, G. H., i
HUESTIS, R. R., 121
HUETTNER, A. F., 121
HUGHES, R. D., 351
HUMPHREY, L. M., 121, 451
HUSKINS, C. L., 508, 510
HUSTED, L., 56, 69, 81
HUTT, F. B., **122**
HUXLEY, J. S., 229, 274, 358
hybridization, sterility, 593
hyperploids, 620
hypoploids, 620

identity of translocations, 118
IMAI, Y., 150, 246
IMMER, F. R., 381
immunology in pigeon species, **114**
inbreeding in cotton, 121
incompatibility
  in Cosmos, **150**–156
  in *Trifolium repens*, 109
Indiana University, 110, 636
interaction of genes, **133**
interference and crossing over, **130**
intergeneric differences in Zea-Euchlaena hybrids, **88**–107
intergeneric hybrid, **134**
intermediate-winged aphids, **134**
inter-relations in toads, **110**
intersexuality, iii, 287
interspecific characters, **128**
interspecific hybridization, 114
inversion, 124, 138
  in Tradescantia, **438**–465
inviability, **134**
Iowa State College, 109, 118, 125, 134, 140
IRWIN, M. R., **114 123, 326**–336
IVES, P. T., 252, 271, 361, 522, 650

JEFFREY, E. C., **124**
John Innes Horticultural Institution, 278
JOLLOS, V., 82
JONES, H. A., 72, 73, 84
JONES, H. E., 178, 181, 183
JONES, D. F., 285, 409, 414, 505
Josiah Macy Jr. Foundation, 391
JUHN, M., 22, 23

KALISS, N., 648
Kansas Agricultural Experiment Station, 129
KANTOR, J. H., **150**
KARPECHENKO, G. D., 2
KARPER, R. E., 387
karyotaxonomy, **131**
KATTERMANN, 402, 405
KATZ, R., 115
KAUFMANN, B. P., 42, 47, 58, 61, 124, 456, 466, 474, 475, 478, 479, 480, 485, 539, 634
KEESLING, H. O., 27

KELLEY, F. J., 16, 18
KEMPTON, J. H., 99, 100
KIHARA, G., 1, 11, 12
KIHARA, H., 402
KIKKAWA, H., 348, 349
KING, R. L., 485
KIRK, L. E., 2
KLINKOWSKI, M., 12
KOBOZIEFF, N., 391, 399
KODANI, M., 584
KOERNER, 575
KOLLER, P. C., 502, 504, 507, 508, 509
KOMATU, Y., 187
KOSTOFF, D., 1, 11, 606, 607, 614, 615
KRAJKA, J., JR., 246, 354
KRAUS, E. J., 614
KRUGER, M., 596
KÜHN, A., 323

LAIBACH, F., 609
LAMAREUX, W. F., **122**
LAMM, R., 453
LAMY, R., 35
LANCEFIELD, R. C., 347
LANDAUER, W., 215, 218, 219, 221, 222
LANDSTEINER, K., 187, 320
LANGHAM, D. G., **88**–107
LARSEN, C. S., 12
larvae
  development of, 111, 143–149
  effect of temperature upon length of life, 231, 241
LAUTERBACH, C. E., 178, 179, 184
LAW, L. W., 339
LAWRENCE, W. J. C., 278
LAWSON, C. A., **125**
leaf variations, **132**
LEBEDEFF, G. A., 409
LECHE, S., 180
LEDINGHAM, G. F., 1–15, 405, 406
LEFF, I. L., **187**–196
left-handedness, 178–186
LEIGHTY, C. E., 405
LEHMAN, E., 150, 154
lethal, 433
  balanced, 636
  in Drosophila, 137
  in house mouse, **391**–400
  in maize, **366**–390
  time of action, 649
LEVAN, A., 310, 507
LEVI, W. M., 25, 35
LEVINE, P., 187
LEVY, H., 542
LEWIS, G. N., 82
LEWIS, J., 278
LEWIS, W. H., 394
LEWITSKY, G. A., 83, 485
LILLIE, F. R., 23, 38
Linaria vulgaris, **140**
LINCOLN, R. E., **125**
LINDSTROM, E. W., 109
linkage
  deer mouse, 110, 121
  fowl, 122
  maize, 95, 299
  Peromyscus, 110, 121
Linum austriacum, 609
Linum perenne, 609
LITTLE, T. M., **150**
LONGLEY, A. E., 12, 402, 511
LOVE, R. M., **126**, 510
LUCE, W. M., **126**, 246, 354
LÜERS, H., 338
Lycopersicon, 596
Lycopersicon esculentum, 595
LYELL, J. C., 24, 35
LYNCH, C. J., 344

MCCLINTOCK, B., 56, 63, 73, 407, 299, 445, 456, 466, 486, 487, 506, 510, 634
MACDOWELL, E. C., 229, 252
MACKENZIE, K., 480, 579
MCNUTT, C. W., **119, 127**
Macrosiphum solanifolii, 289–298
maize, 88–107, **134**, 136, **299**–309, **366**–390, **483**–520
  gametic lethals, **366**–390
  genes
    sugary, 366
    small pollen, 366
    lethal ovule, 366
    Tunicate, 376
    lazy, 376
    Tassel seed-5, 380
    glossy-3, 381
    defective-16, 381
  meiosis, 634
  origin, 106
  translocations, **299**–309
  ultra-violet light, 136
MAKKI, A. I., 576
malformation, 137
MANGELSDORF, P. C., 2, 150, 153, 285, **367**–399, 428
map
  genetic, v
  linkage, in fowl, 122
  linkage, in maize, 299
  salivary, v
MARGOLIS, O. S., 246, 354, 584
MARTIN, C., 31
MAST, S. O., 246
MATHER, K., 45, 47, 56, 69, 75, 82, 282, 285, 448, 451, 452, 453, 455, 460, 467, 470, 474, 485
Medicago hybrids, 1–15
Medicago saliva, 593
METZ, C. W., 61, 62, 84, **127, 128**, 347, 483
METZELAAR, J., 28, 35, 36, 37
METZNER, R., 483
Mexican fresh-water fishes, 118
MICKEY, G. H., 63
microcephaly, 219
MILLER, C. P., 330
MILLER, D. D., 337, 342
MINUTE, 112, 113
minute rearrangements, 566
mitosis, pollen tube of Eschscholtzia, 110
MIYAKE, K., 150
MOHR, O. L., 529
MONTALENTI, G., 22
MORGAN, T. H., i–v, 239
MORGAN, L. V., 510
mosaicism, **16**–40, 630
  in rat, 114
MOSSIGE, J. C., 584, 590

MOTT-SMITH, L. M., 80, 82
mouse
  bifurcated xiphoid, **119**
  changes of dominance in, 116
  coat color, 158
  embryogeny, 394
  genes, 110, 391
  lethal, **391**–400
  linkage, 110, 121
  short ear, 119
  short tail, 116
  tailless, **391**–400
MULLER, H. J., 47, 80, 82, 270, 470, 474, 480, **541**–583, 629, 634
multiple alleles, **120**
MUNRO, S. S., 24, 34
MÜNTZING, A., 11, 84, 402, 406, 454
MUNZ, P. A., 638
MURRAY, M. J., **409**–431
mutation
  in Daphnia, 310–325
  in dioecious plant, 128
  in *Oenothera Lamarckiana*, **135**
  production of, 125, 132
  rate, 46, 125, 132
  wing, 138
Mycodrosophila dimidiata, 351
MYERS, W. M., **126**, **129**

NABOURS, R. K., **129**
NAVASHIN, M., 62, 83, 84
NAWASCHIN, S., 485, 511
NEBEL, B. R., **140**, 310, 451, 453, 506
NEEDHAM, J., 358
NEEL, J. V., **225**–250, **251**–277
Nemesia strumosa, **133**
NEWMAN, H. H., 178, 180, 183
New York Aquarium, 118
New York State Agricultural Experiment Station, 140
New York University, 120, 521
Nicotiana, 153
Nicotiana glutinosa, 595
Nicotiana rustica, 594, 595
Nicotiana tabacum, 596
NISHIYAMA, I., 1, 11, 12
non-disjunction, ii
non-identity of twins, **187**–196
Notch, 115
NOVITSKI, E., 337, 347
nucellus, 593
nuclear fusion, related to chromosomal structure, **124**

OBERLE, G. D., 409
Oenothera, **636**
  balanced lethals, 643
  complexes in, 636
  gamete lethals, 642
  segmental arrangements in, 637
Oenothera biennis, 433, 613, 636
Oenothera blandina, 433
Oenothera cantabrigiana, **433**–437
Oenothera franciscana, 433
Oenothera grandiflora, 642
Oenothera Hookeri, 433, 638
Oenothera irrigua, 639
Oenothera Jamesii, 640
Oenothera laevigata, 642
Oenothera Lamarckiana, **135**, 613
Oenothera longissima, 640
Oenothera muricata, 613
Oenothera nutans, 642
Oenothera Rydbergii, 640
Oenothera strigosa, 640
OFFERMANN, C. A., 62, **130**
Ohio State University, 132, 178
OJEMANN, R. H., 180
OLIVER, C. P., 47, 61, **119**, 546, 575, **584**–592
OLSON, A. R., 82
O'MARA, J. G., **401**–408
Onagra, **636**

Palm dove, 326–336
PANSHIN, I. B., 568, 575
PARK, J. B., 150
PARKER, M. M., **130**
parthenocarpy, 606
PATTERSON, J. D., **131**
PATTERSON, J. T., 338
PEARL, R., 246, 532
Pearlneck, 123, **326**–336
PENG, R. T., 348
Pennsylvania State College, 109, 126, 129
Peromyscus maniculatus, **110**, 121
PETO, F. H., 12, 453
Petunia, 596
Petunia violacea, 595
phenocopies in Drosophila, 111
PHILLIPS, H. M., **131**
Philip, J., 508
photoperiodism, 88
Phytomonas stewartii, 125
pigeon, **16**–40, 114
  cellular characters, 123, 326–336
  mosaicism, **16**–40
pigmentation, guinea pig, 197–214
ploidy, 1, 7, 136, 139
PLOTNIKOWA, T. W., 84
PLOUGH, H. H., 361, 547, 645, 650
PLUNKETT, C. R., 239, 241, 243, 252, 269, 270, 271, 354, 357
pollen, treated with ultra-violet light, **136**
pollen tube, **110**
  cell division, 116
POLLISTER, A. W., 484
polymorphism, 1
polyploidy
  in animals, 310–325
  Melandrium, 139
POMRIASKINSKY-KOBOZIEFF, N. A., 399
POOLE, C. F., 409
POOLE, M., 65
position effect, **111**, **541**
POULSON, D. F., 338, 647
poultry (See fowl)
prime types in Datura, 111
Princeton University, 135
production of "aristapedia," **143**
PROKOFYEVA-BELGOVSKAYA, A. A., 534, 539, 541, 542, 565, 566, 567, 569, 572, 574, 576, 579, 607, 629
pseudo-fertility, 152
pupal period, effect of temperature during, 243

Queens College, 137

rabbit, **127**

RAFFEL, D., **541**–583, 629
RAMSEY, R., 247
RANDOLF, L. F., 429, 507, 510
raspberry, 278–286
rat
  blue mutation, 114
  emotionality, 130
Rattus norvegicus, **114**
REES-LEONARD, O. L., 595, 596
REEVES, R. G., 2, 389, 428
RENNER, O., 376, 613
resistance to fowl paralysis, **139**
RHOADES, M. M., 376, 386, **483**–520
RHOADES, V. H., 376, 386, 511
RICHARDSON, M. M., 454
RICK, C. M., **132, 466**–482
RIEDEL, H., 236
RIFE, D. C., **132, 178**–186
RILEY, H. P., 69, **133,** 467, 471
Ring dove, 123, **326**–336
RIPER, W. VAN, 31
RITTERHAUS, T., 20, 22
ROBERTS, E., 246, 531
ROBERTSON, C. W., 269, 360
ROBERTSON, D. W., 387
ROBINSON, B. A., JR., **150**
Rockefeller Foundation, 638
ROKIZKY, P. TH., 269
ROSA, J. T., 409
ROTHBERG, S., 193
RUDNICK, D., **215**–224
RUSSELL, E. S., 197, 200, 206–211
RUTTLE, M. L., 310, 451, 453
rye, 402

salivary gland chromosomes, v, 618, 622
  chromatic granules in, **127**
  in embryo, 137
  structure, **534**–540
SANDO, W. J., 402
SATINA, S., **133,** 596, 614
SAWIN, P. B., **127**
SAX, K., **41**–68, 69, 70, 71, 73, 75, 79, 85, **134,** 438, 439, 440, 442, 443, 444, 448–453, 455, 459, 462, 463, 466, 467, 470, 471, 474, 475–478, 480, 484
Scaptomyza graminum, 351
SCHAFFNER, J. H., 415
SCHULTZ, J., 239, 252, 270, 534, 539
SCHWAB, J. J., **157**–177
SCHWEITZER, M. D., 648
Sciara ocellaris, 128
SEARS, E. R., **134,** 402, 406, 407
Secale, **401,** 614
Secale cereale, 402
Secale montanum, 402
selective fertilization in raspberries, **278**–286
self-sterility, 150–156
SENN, A. H., 1
SEREBROVSKY, A. S., 20, 21, 541, 562, 568, 571
sex
  balance in aphids, 125
  determination in Amaranthaceae, **409**–431
  differential susceptibility to X-rays, 117
  Drosophila, ii, iii
  mechanism in Melandrium, **139**
sex-linked inheritance, ii
SEYSTER, E. W., 246
SHANK, D. B., **134**
SHARP, L. W., 429, 484
SHRADER, F., 484, 506, 507
SHULL, A. F., **134, 287**–298
SHULL, G. H., **135,** 154
SIDKY, A. R., 576
SINGLETON, W. R., **136, 366**–390
SINITSKAYA, T. G., 542, 577
SINNOTT, E. W., **136**
SIRKS, M. J., 150, 154
SIVERTZEV-DOBZHANSKY, N. P., 648, 650
size differences in plant organs, **136**
skeletal anomalies, 120, 137
SKIRM, G., **136**
SKOLINSKA, M., 12
SLIZYNSKA, H., 387
SLIZYNSKI, B. M., 388
SOBOTTA, J., 394, 398
SOKOLOV, N. N., 480, 571
Solanum tuberosum, 595
somatoplastic sterility, **114, 593**–617
Sonnenblick, B. P., **137**
Sophophora, 337
SPENCER, W. P., 338
SPRAGUE, G. F., 478
speltoid, 126
spontaneous aberrations, **69**–87, **117,** 128, 320
SPRUIJT, C. A. M., 34, 35
STADLER, L. J., 63, 64, 300, 386, 407, 466, 478, 487
STALKER, H., 269
STANDLEY, P. C., 410, 429
STANLEY, W. F., 246, 354, 528, 529, 530, 531, 532, 590
staple length in cotton, 121
STEBBINS, F. M., **129**
STEBBINS, G. L., JR., 444, 448, 451, 452, 454
STEELE, D. G., 16, 17
sterility, 114
  in Cosmos, **150**–156
  pollen, 448
  self-, 462
  somatoplastic, 593
STERN, C., 247, 268, 275, 648
STEVENS, W. L., 284
STILES, K. A., **137,** 287
STONE, W. S., **131,** 485, 546
STOREY, W. B., 409
Storrs Agricultural Experiment Station, 215
Streblomicrodactyly, 120
STREET, J. C., 85
Streptopelia chinensis, 326–336
Streptopelia risoria, 326–336
Streptopelia senegalensis, 326–336
STURTEVANT, A. H., 84, 252, 254, 270, **337**–353, 454, 455, 459, 460, 463, 650
susceptibility to X-rays, 60, 117
SUTTON, I., 150, 466, 478, 480, **534**–540, 618, 620, 621, **628**–635
SWANSON, C. P., **138, 438**–465

TABUCHI, K., 150
TAN, C. C., 346, 349
TAYLOR, J. W., 405
telomere, 634
temperature
  effect on development, 225–250
  effect on vestigial, **119**
  effect on wing size in Drosophila, **521**–533
temperature-effective period, 354–365

teosinte, 88–107
TERAO, A., 150, 154
tetraploidy
  Medicago, 1, 7
  Tradescantia, 136
tetrosomic inheritance in Dactylis, **126**
TETREAULT, T. E., **127**
Texas Agricultural Experiment Station, 366
THOMPSON, D'ARCY, 274
THOMPSON, W. P., 1, 11, 12, 402, 405, 406, 612, 614
THOMSEN, O., 193
TIMOFEEFF-RESSOVSKY, H. A., 270, 273
TIMOFEEFF-RESSOVSKY, N. W., 46, 60, 64, 79, 80, 82, 157, 270, 273, 478, 479, 480, 575
TINIAKOV, G. G., 571
toad, **110**
top-root ratios in maize, **134**
Tradescantia
  colchicine treated cells, 116
  induced deletions, 466
  induced mutations, 41–68, 132
  inversions, **138, 438**
  quadrivalent formation, **136**
  spontaneous mutations, 69–87, 117
Tradescantia bracteata, 455
Tradescantia canaliculata, 439
Tradescantia humilis, 439
Tradescantia paludosa, 455
Tradescantia rosea, 459
Tradescantia virginiana, 455
TRANKOWSKY, D. A., 484
translocation
  genetic identity of, **118**
  in maize, 299–309
transplantation
  antennal disks, 143–149
  eye disks, 112, 146
triploidy, Medicago, 6
trisomes, secondary, origin, 508
Triticale, **401**–408
Triticum, **401**, 402, 614
Triticum monococcum, 134
Triticum vulgare, 402
twins, **178**, 186, 187–196
TYSDAL, H. M., 1

UBER, F. M., 65, 467
ultra-violet light, treatment of maize pollen, 136
University
  Alabama, 110
  Arkansas, 121
  California, 111, 143
  Chicago, 130, 157, 197
  Edinburgh, 541
  Michigan, 110, 134, 287, 433
  Minnesota, 119, 303, 584
  Missouri, 134
  Oklahoma, 116
  Rochester, 225
  Texas, 131
  Toronto, 140
  Washington, 133
  Wisconsin, 1, 16, 113, 114, 123, 138, 299, 326, 593
U. S. Department of Agriculture, 115, 134, 401, 483
U. S. Regional Poultry Research Laboratory, 139
UPCOTT, M., 72, 73, 457, 459, 485, 502, 504, 507, 508, 509

variation, hereditary, 127
vena cava in rabbit, 127
VERSCHUER, O., 179
viability
  Drosophila, 157
  poultry, **139**
virilis complex in Drosophila, **131**
virulence, **140**
VOGT, 575

WAKAKUWA, SH., 1, 11
WALDRON, L. R., 1
WALETZKY, E., **138**
WALKER, R. I., 310
WARD, E., 246
WARMKE, H. E., **139**
WARREN, D. C., 158
Washington Square College, 120, 521
Washington University, 215
WATERS, N. F., **139**
WATKINS, A. E., 11
WESTERGAARD, M., 12
WESTOVER, H. L., 1
wheat (See Triticum)
WHALEY, W. G., **136**
WHITAKER, T. W., 71, 450
WHITE, M. J. D., 452
WHITNEY, R., 321
WIENER, A. S., **187**–196, 330
WILSON, E. B., ii
WILSON, G. B., **140**
WILSON, P. T., 178, 181, 183
wing, 120, **287**–298
  bifid, 138
  development, 120, 125, **134**
  form, 120
  Notch, 115
  size, 120
  vestigial, 119
wingedness, 287–298
Wittenberg College, 125
WOOD, T. R., 318
WOODSIDE, G. L., 650
WOODSON, R. E., 439
WOOLLEY, G. W., 326
WORSAAL, E., 193
WRIEDT, C., 17, 18
WRIGHT, A. H., 1
WRIGHT, E. S., 394
WRIGHT, G., **140**
WRIGHT, S., 35, 176, 197, 198
Wrinkled, **138**

X-chromosome, ii
  left limb, 338
  right limb, 339
  linkage map, 344
X-rays, 41–68, 69, 81
  differential susceptibility to, 60, 117
  induction of chromosome aberrations, 41–68, 124, 129, 132, 134
  induction of chromosome deletions, 466–482
  mutations, 125
  Notch, 115

sensitivity to, 140
treatment of Drosophila, 111, 115

YAMPOLSKY, C., 409
YARNELL, S. A., 150, 154

yellow series, coat color, 199, 204–209
Zea mays, telocentric chromosome, 483–520
ZELENY, C., 246
ZELLE, M. R., 140

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TABLE OF CONTENTS

JANUARY NUMBER

PAGE

LEDINGHAM, G. F., Cytological and developmental studies of hybrids between *Medicago sativa* and a diploid form of *M. falcata*. 1

HOLLANDER, W. F., AND COLE, L. J., Somatic mosaics in the domestic pigeon. 16

SAX, KARL, An analysis of X-ray induced chromosomal aberrations in Tradescantia. 41

GILES, NORMAN, Spontaneous chromosome aberrations in Tradescantia. 69

LANGHAM, D. G., The inheritance of intergeneric differences in Zea-Euchlaena hybrids. 88

Abstracts of papers, presented at the 1939 Meetings of the Genetics Society of America. 109

MARCH NUMBER

BRAUN, WERNER, Experimental evidence on the production of the mutant "aristapedia" by a change of developmental velocities. 143

LITTLE, T. M., KANTOR, J. H., AND ROBINSON, B.A., JR., Incompatibility studies in *Cosmos bipinnatus*. 150

SCHWAB, JOSEPH J., A study of the effects of a random group of genes on shape of spermatheca in *Drosophila melanogaster*. 157

RIFE, D. C., Handedness, with special reference to twins. 178

WIENER, ALEXANDER S., AND LEFF, IRVING L., Chances of establishing the non-identity of biovular twins, with special reference to individuality tests of the blood. 187

HEIDENTHAL, GERTRUDE, A. cholorimetric study of genic effect on guinea-pig coat color. 197

RUDNICK, DOROTHEA, AND HAMBURGER, VIKTOR, On the identification of segregated phenotypes in progeny from Creeper fowl matings. 215

NEEL, JAMES V., The interrelations of temperature, body size, and character expression in *Drosophila melanogaster*. 225

MAY NUMBER

NEEL, JAMES V., The pattern of supernumerary machrochaetae in certain Drosophila mutants. 251

TABLE OF CONTENTS

(CONTINUED)

PAGE

LEWIS, D., Genetical studies in cultivated raspberries. II. Selective fertilization 278

SHULL, A. FRANKLIN, Adult intermediate-winged aphids not physiologically intermediate 287

ANDERSON, E. G., AND BRINK, R. A., Translocations in maize involving chromosome 3 299

DUNHAM, H. HOWARD, AND BANTA, A. M., Some effects of colchicine on heredity in *Daphnia longispina* 310

IRWIN, M. R., AND COLE, L. J., Further studies of the interrelationships of cellular characters in Columbidae 326

JULY NUMBER

STURTEVANT, A. H., Genetic data on *Drosophila affinis*, with a discussion of the relationships in the subgenus Sophophora ... 337

CHILD, GEORGE, An analysis of the bristle forming reactions in *Drosophila melanogaster* scute-1 354

SINGLETON, W. R., AND MANGELSDORF, P. C., Gametic lethals on the fourth chromosome of maize 366

GLUECKSOHN-SCHOENHEIMER, S., The effect of an early lethal (l^0) in the house mouse 391

O'MARA, J. G., Cytogenetic studies on Triticale. I. A method for determining the effects of individual Secale chromosomes on Triticum 401

MURRAY, M. J., The genetics of sex determination in the family Amaranthaceae 409

SEPTEMBER NUMBER

DAVIS, B. M., *Oenothera cantabrigiana* 433

SWANSON, C. P., The distribution of inversions in Tradescantia 438

RICK, C. M., On the nature of X-ray induced deletions in Tradescantia chromosomes 466

RHOADES, M. M., Studies of a telocentric chromosome in maize with reference to the stability of its centromere 483

HARNLY, M. H., The temperature responses of flies with the deficiency vestigial-depilate in *Drosophila melanogaster* 521

SUTTON, EILEEN, The structure of salivary gland chromosomes of *Drosophila melanogaster* in exchanges between euchromatin and heterochromatin 534

TABLE OF CONTENTS
(CONTINUED)

NOVEMBER NUMBER

PAGE

RAFFEL, DANIEL, AND MULLER, H. J., Position effect and gene divisibility considered in connection with three strikingly similar scute mutations 541

GREEN, M. M., AND OLIVER, C. P., The action of certain mutants upon the penetrance of heterozygous vestigial wing in *Drosophila melanogaster* 584

COOPER, D. C., AND BRINK, R. A., Somatoplastic sterility as a cause of seed failure after interspecific hybridization 593

DEMEREC, M., Genetic behavior of euchromatic segments inserted into heterochromatin 618

SUTTON, EILEEN, Terminal deficiencies in the X-chromosome of *Drosophila melanogaster* 628

CLELAND, RALPH E., Analysis of wild American races of Oenothera (Onagra) 636

BRODY, GEORGE, The time of effect of some second chromosome lethals in *Drosophila melanogaster* 645

Index to Volume 25 651

CORRIGENDA

VOLUME 25, 1940

Page 26, line 12, for "B?/B^{A} D; C/c" read "Bd/B^{A} D; C/c."

Page 30, figure 10, for "left side blue checker, *S*" read "left side blue checker, *s*."

Page 195, line 2 in "Addendum," for "(1939)" read "(1938)."

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GENETICS Vol. 25 No. 6 NOVEMBER, 1940

CONTENTS OF PRECEDING ISSUES

JANUARY, 1940

LEDINGHAM, G. F., Cytological and developmental studies of hybrids between *Medicago sativa* and a diploid form of *M. falcata*.
HOLLANDER, W. F., AND COLE, L. J., Somatic mosaics in the domestic pigeon.
SAX, KARL, An analysis of X-ray induced chromosomal aberrations in Tradescantia.
GILES, NORMAN, Spontaneous chromosome aberrations in Tradescantia.
LANGHAM, D. C., The inheritance of intergeneric differences in Zea-Euchlaena hybrids.
Abstracts of papers, presented at the 1939 Meetings of the Genetics Society of America.

MARCH, 1940

BRAUN, WERNER, Experimental evidence on the production of the mutant "aristapedia" by a change of developmental velocities.
LITTLE, T. M., KANTOR, J. H., and ROBINSON, B. A., Jr., Incompatibility studies in *Cosmos bipinnatus*.
SCHWAB, JOSEPH J., A study of the effects of a random group of genes on shape of spermatheca in *Drosophila melanogaster*.
RIFE, D. C., Handedness, with special reference to twins.
WIENER, ALEXANDER S., and LEFF, IRVING L., Chances of establishing the non-identity of biovular twins, with special reference to individuality tests of the blood.
HEIDENTHAL, GERTRUDE, A colorimetric study of genic effect on guinea-pig coat color.
RUDNICK, DOROTHEA, and HAMBURGER, VIKTOR, On the identification of segregated phenotypes in progeny from Creeper fowl matings.
NEEL, JAMES V., The interrelations of temperature, body size, and character expression in *Drosophila melanogaster*.

MAY, 1940

NEEL, JAMES V., The pattern of supernumerary machrochaetae in certain Drosophila mutants.
LEWIS, D., Genetical studies in cultivated raspberries. II. Selective fertilization.
SHULL, A. FRANKLIN, Adult intermediate-winged aphids not physiologically intermediate.
ANDERSON, E. G., and BRINK, R. A., Translocations in maize involving chromosome 3.
DUNHAM, H. HOWARD, and BANTA, A. M., Some effects of colchicine on heredity in *Daphnia longispina*.
IRWIN, M. R., and COLE, L. J. Further studies of the interrelationships of cellular characters in Columbidae.

JULY, 1940

STURTEVANT, A. H., Genetic data on *Drosophila affinis*, with a discussion of the relationships in the subgenus Sophophora.
CHILD, GEORGE, An analysis of the bristle forming reactions in *Drosophila melanogaster* scute-1.
SINGLETON, W. R., and MANGELSDORF, P. C., Gametic lethals on the fourth chromosome of maize.
GLUECKSOHN-SCHOENHEIMER, S., The effect of an early lethal (t^9) in the house mouse.
O'MARA, J. G., Cytogenetic studies on Triticale. I. A method for determining the effects of individual Secale chromosomes on Triticum.
MURRAY, M. J., The genetics of sex determination in the family Amaranthaceae.

SEPTEMBER, 1940

DAVIS, B. M., *Oenothera cantabrigiana*.
SWANSON, C. P., The distribution of inversions in Tradescantia.
RICK, C. M., On the nature of X-ray induced deletions in Tradescantia chromosomes.
RHOADES, M. M., Studies of a telocentric chromosome in maize with reference to the stability of its centromere.
HARNLY, M. H., The temperature responses of flies with the deficiency vestigial-depilate in *Drosophila melanogaster*.
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